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COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council
Good Chemistry Makes it Possible

November 6, 2000

Charles M. Auer
Director, Chemical Control Division
Office of Pollution Prevention and Toxics
US Environmental Protection Agency
401 M Street, S.W., MC 7405
Washington, DC 20460

RE: Test Plan for C5 Non-Cyclics Category

Dear Mr. Auer:

Enclosed is the test plan for the Olefins Panel's C5 Non-Cyclics Test Category under the HPV Chemical Challenge Program. The members of the Panel are listed in the test plan. Also enclosed are robust summaries for existing data for the SIDS endpoints on substances included in the category. The test plan and robust summaries will also be submitted to you electronically.

If you have any questions, please call Dr. Elizabeth Moran, manager of the Olefins Panel, at 301/924-2006.

Sincerely yours,

HIGH PRODUCTION VOLUME (HPV)

CHEMICAL CHALLENGE PROGRAM

TEST PLAN

For The

C5 Non-Cyclics Category

Prepared by:

American Chemistry Council
Olefins Panel
HPV Implementation Task Group

November 6, 2000

PLAIN ENGLISH SUMMARY

This test plan addresses streams which are products of the ethylene process and associated C5 processes and which contain, predominantly, isoprene and/or other C5 or C6 non-cyclic alkenes and alkanes. The plan addresses the category by evaluating four substances: pure isoprene (data are already available except for aquatic toxicity data - an acute algal toxicity study will be conducted), a mid-range isoprene stream containing 14-20 percent isoprene (testing will be conducted), a low concentration stream with approximately 2 percent isoprene (testing will be conducted), and a high-purity 2-methyl-2-butene stream (testing will be conducted). The test plan is based on the expectation that the presence of isoprene and 2-methyl-2-butene will be responsible for the biological activity of the streams. This assumption is based in part on existing data for isoprene and 2-methyl-2-butene, and also on what is known about the other components. Isoprene and 2-methyl-2-butene are sponsored through the ICCA (International Council of Chemical Associations) HPV Program. Additional supporting data will be collected on many of the other components as part of other test plans under the HPV Challenge Program, the ICCA program, or from chemicals already sponsored in the OECD SIDS program.

EXECUTIVE SUMMARY

The Olefins Panel (Panel) of the American Chemistry Council and the Panel's member companies hereby submit for review and public comment the test plan for the C5 Non-Cyclics category under the Environmental Protection Agency's (EPA) High Production Volume (HPV) Chemical Challenge Program. It is the intent of the Panel and its member companies to use new information in conjunction with a variety of existing data and scientific judgment/analyses to adequately characterize the SIDS (Screening Information Data Set) human health, environmental fate and effects, and physicochemical endpoints for this category.

This test plan addresses streams which are products of the ethylene process and associated C5 processes and which contain, predominantly, isoprene and/or other C5 or C6 non-cyclic alkenes and alkanes. The plan addresses the category by evaluating four substances:

- Isoprene

  This is a high purity isoprene stream. Data for HPV endpoints are already available except for aquatic toxicity. An acute algal toxicity study, OECD Guideline 201, will be conducted. In addition, structural activity relationships will be used to predict toxicity to fish and *Daphnia*. Isoprene is sponsored through the ICCA (International Council of Chemical Associations) HPV Program.

- Pyrolysis C5s

  This is a mid-range isoprene stream containing 14-20 percent isoprene. The following studies will be conducted: An Ames test (OECD Guideline 471), a mouse inhalation micronucleus test (OECD Guideline 474), a rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422), an algal toxicity test (OECD Guideline 201), a *Daphnia* sp. acute toxicity test (OECD Guideline 202), a fish acute toxicity test (OECD Guideline 203), and a biodegradation test (OECD Guideline 301F).

- Hydrotreated C5s

  This is a low isoprene stream containing approximately 2 percent isoprene. The following studies will be conducted: An Ames test (OECD Guideline 471), a mouse inhalation micronucleus test (OECD Guideline 474), a rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422), an algal toxicity test (OECD Guideline 201), a *Daphnia* sp. acute toxicity test (OECD Guideline 202), a fish acute toxicity test (OECD Guideline 203), and a biodegradation test (OECD Guideline 301F).

- 2-Methyl-2-Butene

This is a high purity 2-methyl-2-butene stream. Data are already available for genetic toxicity. The following studies will be conducted: A rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422), an algal toxicity test (OECD Guideline 201), a *Daphnia* sp. acute toxicity test (OECD Guideline 202), a fish acute toxicity test (OECD Guideline 203), and a biodegradation test (OECD Guideline 301F). 2-Methyl-2-butene is sponsored through the ICCA HPV Program.

The test plan is based on the expectation that the presence of isoprene and 2-methyl-2-butene will be responsible for the biological activity of the streams. This assumption is based in part on existing data for isoprene and 2-methyl-2-butene, and also on what is known about the other components. Additional supporting data will be collected on many of the other components as part of other test plans under the HPV Challenge Program, the ICCA program, or from chemicals already sponsored in the OECD SIDS program.

Predictive computer models will be used to develop relevant environmental fate and physiocochemical data for substances in the C5 Non-Cyclics category. Environmental fate information will be summarized either through the use of computer models when meaningful projections can be developed or in technical discussions when computer modeling is not applicable. For mixed streams, physicochemical properties will be represented as a range of values according to component composition. These data will be calculated using a computer model cited in an EPA guidance document prepared for the HPV Challenge Program.

LIST OF MEMBER COMPANIES
THE OLEFINS PANEL

The Olefins Panel includes the following member companies:

BP Amoco Chemicals
Chevron Phillips Chemical Company
CONDEA Vista Company*
The Dow Chemical Company
E. I. du Pont de Nemours and Company*
Eastman Chemical Company*
Equistar Chemicals, LP
ExxonMobil Chemical Company
Fina Oil and Chemical Company*
Formosa Plastics Corporation, U.S.A.*
The B.F.Goodrich Company*
The Goodyear Tire & Rubber Company
Huntsman Corporation
Koch Industries*
NOVA Chemicals Inc.
Shell Chemical Company
Sunoco, Inc.*
Texas Petrochemicals Corporation*
Union Carbide Corporation*
Westlake Chemical Corporation*
Williams Olefins, LLC*

* These companies are part of the Olefins Panel but do not produce streams in the C5 Non-Cyclics Category.

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TEST PLAN FOR THE C5 NON-CYCLICS CATEGORY

I. INTRODUCTION

The Olefins Panel (Panel) of the American Chemistry Council and the Panel's member companies have committed to develop screening level human health effects, environmental effects and fate, and physicochemical data for the C5 Non-Cyclics category under the Environmental Protection Agency's (EPA's) High Production Volume (HPV) Challenge Program (Program).

This plan identifies CAS numbers used to describe process streams in the category, identifies existing data of adequate quality for substances included in the category, and outlines testing needed to develop screening level data for this category under the Program. This document also provides the testing rationale for the C5 Non-Cyclics category. The objective of this effort is to identify and develop sufficient test data and/or other information to adequately characterize the human and environmental health and environmental fate for the category in compliance with the EPA HPV Program. Physicochemical data that are requested in this program will be calculated as described in EPA guidance documents.

II. DESCRIPTION FOR THE C5 NON-CYCLICS CATEGORY

A. The Category

The C5 Non-Cyclics category was developed by grouping ethylene manufacturing streams that the Panel believes are similar from both a process and a toxicology perspective, which is why this group is considered a category for purposes of the HPV Program. Sixteen CAS numbers (Table 1) are used to describe these eleven process streams arising from the ethylene process and other associated C5 processes. Nine of these process streams are complex reaction products. The CAS numbers used to represent these nine products are generally vague with respect to the specifics that distinguish the streams within the category. Therefore a single stream is correctly represented by more than one CAS number and a CAS number may be applicable to more than one stream. A process stream is a mixture of chemicals that arises from a chemical reaction or separation activity. A description of the ethylene and associated processes is included in Appendix I.

The streams in this category consist of high purity hydrocarbons and complex hydrocarbon reaction products that contain significant levels of olefins with a carbon number distribution that is predominantly C5 or C6. The typical compositions of the streams are shown in Table 2. All but three of these streams contain isoprene. The three streams that do not contain isoprene consist of C5 and/or C6 alkenes that

are predicted to have a toxicology profile similar to that of the 2% isoprene stream. Typically, only six of the many components of the streams (isopentane, isoprene, pentane, 2-methyl-2-butene, neohexene, 1,3-pentadiene) are present at concentrations ≥30%; and only six more components (2-butene, isopentene, 2-pentene, cyclopentadiene, cyclopentene, methyl-pentenes-2) are present at ≥20%. The category is designated C5 Non-Cyclics.

The CAS Numbers in the C5 Non-Cyclics category are associated with eleven streams which are commercial products or isolated intermediates:

1. Pyrolysis C5s
2. Hydrotreated C5s
3. Pentenes
4. Piperylene Concentrate
5. Isoprene Concentrate
6. Isoprene-Piperylene Concentrate
7. Isoprene, High Purity
8. Isoprene Purification Byproduct
9. 2-Methyl-2-Butene
10. Metathesis Byproduct
11. Neohexene

Descriptions of the eleven streams associated with the C5 Non-Cyclics category are presented below:

1. Pyrolysis C5s

Pyrolysis C5s (or C5 fraction) consist of a hydrocarbon distillate fraction separated from pyrolysis gasoline (the C5+ portion of the cracked gas in the ethylene process). The carbon number distribution of the product is predominantly C5, but the stream also typically contains relatively low levels of the higher boiling C4 substances (e.g. 1,2-butadiene) as well as low levels of the more volatile C6 hydrocarbons. Benzene content is typically 0.25% and present in the distillate largely due to azeotropes of benzene with other hydrocarbon species in the complex mixture. The 1,3-butadiene content is typically 1%. The stream contains significant levels of olefins, diolefins and cyclics.

2. Hydrotreated C5s

Hydrotreated C5s result from hydrogenation of Pyrolysis C5s over catalyst. Typically the stream that is charged to the hydrogenation reactor is a broader boiling range stream than the C5 fraction. For example, a full range pyrolysis gasoline may be hydrotreated and the resulting product then fractionated to produce the Hydrotreated C5s as a distillate fraction. The hydrogenation process may be either a one-stage or two-stage process. The one-stage process is typically a liquid-phase process where the primary objective is to selectively convert diolefins to monoolefins. The two-stage process is typically a vapor-phase, more severe hydrogenation that converts monoolefins to paraffins. Typically,

Hydrotreated C5s are subject only to one-stage hydrogenation because the product is intended for use in gasoline where the monoolefins are desired components. Similar to Pyrolysis C5s, Hydrotreated C5s have a carbon number distribution that is predominantly C5, and contain low levels of the higher boiling C4 substances as well as low levels of the more volatile C6 hydrocarbons. Benzene content is typically 1%. Unlike pyrolysis C5s, the diolefin content in Hydrotreated C5s is very low.

3. Pentenes

Pyrolysis C5s are typically fractionated into concentrates of the reactive diolefins: isoprene, piperylene (1,3-pentadiene) and cyclopentadiene (as dimer.) As a first step in producing these concentrates, the lighter boiling fraction of the stream, i.e., the compounds that are more volatile than isoprene, are sometimes removed as a distillate. This distillate is designated as Pentenes or the Pentenes Cut. The stream has a carbon number distribution that is predominantly C4-C5, consisting in part of iso-pentane and the more volatile pentenes such as 1-pentene, with about 1-3% isoprene. The stream typically contains the C4 compounds that were present in the Pyrolysis C5s, including 1,3-butadiene. Alternately, Pentenes can be removed later in processing, for example by distillation of the Isoprene Concentrate.

4. Piperylene Concentrate

Production of Piperylene Concentrate (cis- and trans-1,3-pentadiene) from Pyrolysis C5s is accomplished by first "heat soaking" the stream in order to dimerize 1,3-cyclopentadiene (CPD). This is necessary because the boiling point of CPD is within 2.5 °F of that of trans-1,3 pentadiene. The heat soak produces a mixture of CPD dimer and codimers (DCPD Concentrate) that can be removed as a bottoms product from the balance of the Pyrolysis C5 stream. After removal of the DCPD Concentrate, what is left of the Pyrolysis C5s can be charged to a distillation column (the isoprene-piperylene splitter) to yield Piperylene Concentrate as a bottoms product. The carbon number distribution for Piperylene concentrate is predominantly C5. A typical Piperylene Concentrate stream composition includes 60% piperylenes, 10% 2-methyl-2-butene, and about 0.2% benzene.

5. Isoprene Concentrate

The isoprene-piperylene splitter described for the above stream also yields Isoprene Concentrate as a distillate. The carbon number distribution for Isoprene concentrate is predominantly C5. A typical Isoprene Concentrate stream contains 40% isoprene with the balance largely iso- and n-pentane and C5 monolefins. Pentenes, as described for the Pentenes stream, may or may not have been removed in the distillation sequence and this has the corresponding effect on the concentration of the lower boiling pentene and pentane components in the Isoprene Concentrate.

6. Isoprene-Piperylene Concentrate

The intermediate process stream charged to the isoprene-piperylene splitter (as described above for piperylene concentrate) is sometimes isolated as a product. This stream typically contains about 20% isoprene and 14% piperylenes.

7. Isoprene, High Purity

High purity isoprene (98+%) is produced by separation from isoprene concentrate. This is accomplished using an extractive distillation process.

8. Isoprene Purification Byproduct

Isoprene Purification Byproduct is a byproduct from the Isoprene purification process.
The carbon number of the stream is predominantly C5 and the composition is largely iso- and n-pentane, plus lesser amounts of pentenes and about 5% isoprene. The byproduct may also contain 1,3-butadiene at about 0.5%.

9. 2-Methyl-2-Butene

The component 2-methyl-2-butene is sometimes separated from a mixed C5 stream by first converting to an intermediate, then separating the intermediate from the mix by distillation, and then cracking the intermediate back to yield product 2-methyl-2-butene.

10. Metathesis Byproduct

An olefins plant may include a Metathesis process which converts ethylene and/or butenes into propylene. This process produces a byproduct (referred to here as Metathesis Byproduct). The stream is a gasoline stream consisting primarily of C5 and C6 olefins.

11. Neohexene

Neohexene is a high purity product (typically 97% 3,3-dimethyl-1-butene), derived by reaction of diisobutylene and ethylene.

III. TEST PLAN RATIONALE

A. Overview

Human Health Effects

In addition to a nearly complete HPV SIDS (Screening Information Data Sets) data set for isoprene and genetic toxicity data for 2-methyl-2-butene, a substantial amount of toxicity data are available for

many of the other components of the streams in the C5 Non-Cyclics category. Some of the components are SIDS materials, and some components will be tested by the American Chemistry Council Olefins Panel within other category test plans or by other groups within the HPV or ICCA programs.

Based on examination of existing data for components of the streams in the C5 Non-Cyclics category, isoprene and, to a lesser extent, 2-methyl-2-butene are expected to be the most biologically active of the major components in the category and thus the major contributors to toxicological activity, with genotoxicity the endpoint of concern. Of the SIDS endpoints, only the genetic toxicity tests are known to show a dose-related adverse response with isoprene. With the exception of acute central nervous system effects at high concentrations, none of the other components that are present in substantial amounts in these streams has demonstrated a potential to cause significant adverse health effects.

It is anticipated that the biological spectrum of activity for isoprene, with regard to positive genetic toxicity, may be reflected in other streams in this category that contain isoprene. However, since metabolism of isoprene is required for toxicity, and other C5 alkenes are metabolized through a common metabolic pathway, it is anticipated that mixed components will compete for the same active enzyme sites. Different individual toxicities, which are dependent on the formation of biologically active metabolites, may be reduced, as less metabolite(s) will be produced through competition for these sites. Hence the positive genotoxicity of isoprene, or the less potent 2-methyl-2-butene, may in fact be reduced or eliminated by the greater presence of the other components. This can only be assessed by testing a mixed stream.

Thus, the strategy for characterizing the hazards of this group is based on testing a representative product with mid-range isoprene content (14-20%) and one with low isoprene content (approximately 2%) in full SIDS human health test batteries (except for acute inhalation toxicity which is not deemed informative for the HPV Challenge Program). The following tests will be conducted: A bacterial gene mutation test (Ames test, OECD Guideline 471), a mouse inhalation micronucleus test for chromosome aberrations (OECD Guideline 474), and a rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422). This strategy will allow an evaluation of the impact of isoprene on the toxicity of the mixed streams, and will also allow an assessment of the hazards of the other components when the influence of isoprene is reduced or eliminated. The streams tested will include other chemical components representative of other streams that make up this category. The exact composition of the streams to be tested will be determined analytically at the time of testing. In addition, the SIDS human health data set (except for the acute inhalation toxicity test which is not deemed informative for the HPV Challenge Program) will be completed for 2-methyl-2-butene. The following tests will be conducted: A bacterial gene mutation test (Ames test, OECD Guideline 471), a mouse inhalation micronucleus test for chromosome aberrations (OECD Guideline 474), a rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422). 2-Methyl-2-butene is sponsored through the ICCA HPV Program.

The inhalation route of exposure was chosen for the health effects testing because inhalation is the most

relevant route of exposure for the C5 Non-Cyclics streams. The mouse micronucleus test was chosen for chromosomal effects testing because isoprene is negative in *in vitro* tests but positive in the mouse micronucleus test. 2-Methyl-2-butene is also positive in the mouse micronucleus test. The mouse is the standard species for micronucleus tests and a substantial historical database exists for the mouse in this test. The rat will be used in the repeated dose/reproductive and developmental effects/neurotoxicity screen because this test was designed for the rat and there is a historical data base for the rat but not for the mouse. The rat is also the standard species for reproductive toxicity tests. Furthermore, there is a substantial amount of data developed in rats, mice, primates, and humans (*in vitro*) providing strong support for the proposition that the rat is a scientifically more appropriate model for humans than is the mouse.

The recommended testing, together with existing data and data for the components under development by the American Chemistry Council Olefins Panel for other categories under the HPV program, by other HPV consortia, and by the OECD SIDS program, will be sufficient to adequately characterize the toxicity of the range of substances included in this category.

Physical-Chemical Properties

Physicochemical data for each of the 11 streams in the C5 Non-Cyclics category will be developed using the EPIWIN© model[1], as discussed in the EPA document titled "The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program."

Ecotoxicity

The product streams of this category are expected to cause similar moderate acute aquatic toxicity to freshwater fish and invertebrates and moderate toxicity to freshwater algae. This is based on existing data for similar saturated hydrocarbons and results of computer modeling using ECOSAR for selected chemical components of this category [ECOSAR is an aquatic toxicity modeling program and is a subroutine contained in EPIWIN[1]]. In addition, isoprene and 2-methyl-2-butene, which are contained by several streams in this category, are also expected to exhibit a similar degree of toxicity as the streams. To demonstrate the expected toxic effects in each of three aquatic organisms, the Panel will test the following streams and chemicals:

- Pyrolysis C5s: A product stream with mid-range isoprene content (approximately 14-20%);
- Hydrotreated C5s: A product stream with low isoprene content (approximately 2%);
- 2-methyl-2-butene (sponsored through ICCA HPV Program); and
- isoprene (sponsored through ICCA HPV Program).

The testing for all these materials except isoprene will include an alga toxicity test (OECD Guideline 201), a *Daphnia* sp. acute toxicity test (OECD Guideline 202), and a fish acute toxicity test (OECD Guideline 203). Because isoprene is contained in the two streams, the Panel will also conduct one test, an algal toxicity test, with a high purity isoprene to confirm that the results are similar to the two tested

streams and 2-methyl-2-butene. An alga was selected because it was calculated by ECOSAR to be the more sensitive organism of the three aquatic trophic levels included in the HPV Program. In addition, structural activity relationships will be used to predict toxicity to fish and *Daphnia*.

Environmental Fate

A biodegradation test via manometric respirometry (OECD Guideline 301F) will be conducted on a representative product with mid-range isoprene content (approximately 14-20%), one with low isoprene content (approximately 2%), and 2-methyl-2-butene. This test guideline uses a closed test system, which is required when assessing the biodegradation of volatile materials like those in this category. It is also recommended when evaluating mixtures containing several chemical species, some of which may have minimally water-soluble components.

The endpoints for photodegradation, hydrolysis, transport, and fugacity will be either calculated or discussed. Chemical equilibrium models are used to calculate fugacity, which is only calculated. Chemical components of process streams in the C5 Non-Cyclics category are calculated to partition primarily to the air, and therefore their fate in air is of environmental interest (this is discussed below under photodegradation). In addition, these components have relatively low Kow values, which suggest that they will not tend to partition to suspended organic matter in air and precipitate to aquatic and terrestrial compartments.

1. Photodegradation – Photolysis

Direct photochemical degradation occurs through the absorbance of solar radiation by a chemical substance. If the absorbed energy is high enough, then the resultant excited state of the chemical may undergo a transformation. Simple chemical structures can be examined to determine whether a chemical has the potential for direct photolysis in water. First order reaction rates can be calculated for some chemicals that have a potential for direct photolysis using the procedures of Zepp and Cline[2]. UV light absorption of the 11 streams in the category will be evaluated to identify those having the potential to degrade in solution. For those compounds with a potential for direct photolysis in water, first order reaction rates will be calculated.

2. Photodegradation – Atmospheric Oxidation

Photodegradation can be measured[3] (EPA identifies OECD test guideline 113 as a test method) or estimated using models accepted by the EPA[4]. An estimation method accepted by the EPA includes the calculation of atmospheric oxidation potential (AOP). Atmospheric oxidation as a result of hydroxyl radical attack is not direct photochemical degradation, but rather indirect degradation. AOPs can be calculated using a computer model. Light hydrocarbons, such as those in the C5 Non-Cyclics category, readily volatilize to air. In air, chemicals may undergo reaction with photosensitized oxygen in the form of ozone and hydroxyl radicals. The computer program AOPWIN (atmospheric oxidation program for Microsoft Windows)[1] is used by OPPTS (Office of Pollution Prevention and Toxic Substances). This program calculates a chemical half-life based on an overall OH reaction rate constant, a 12-hr day, and a given OH concentration. This calculation will be performed for the representative components of the 11 streams in the C5 Non-Cyclics category.

3. Stability in Water (Hydrolysis Testing and Modeling)

Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters[5]. Stability in water can be measured[3] (EPA identifies OECD test guideline 111 as a test method) or estimated using models accepted by the EPA[4]. An estimation method accepted by the EPA includes a model that can calculate hydrolysis rate constants for esters, carbamates, epoxides, halomethanes, and selected alkylhalides. The computer program HYDROWIN (aqueous hydrolysis rate program for Microsoft windows)[1] is used by OPPTS.

All of the chemical structures included in the C5 Non-Cyclics category are simple hydrocarbons. That is, they consist entirely of carbon and hydrogen. As such they are not expected to hydrolyze at a measurable rate. A technical document will be prepared describing the potential hydrolysis rates of these substances, the nature of the chemical bonds present, and the potential reactivity of this class of chemicals with water.

4. Chemical Transport and Distribution In The Environment (Fugacity Modeling)

Fugacity based multimedia modeling can provide basic information on the relative distribution of chemicals between selected environmental compartments (i.e., air, soil, sediment, suspended sediment, water, biota). The US EPA has acknowledged that computer modeling techniques are an appropriate approach to estimating chemical partitioning (fugacity is a calculated endpoint and is not measured). A widely used fugacity model is the EQC (Equilibrium Criterion) model[6]. EPA cites the use of this model in its document titled *Determining the Adequacy of Existing Data*[3], which was prepared as guidance for the HPV Program.

In its document, EPA states that it accepts Level I fugacity data as an estimate of chemical distribution values. The input data required to run a Level I model include basic physicochemical parameters; distribution is calculated as percent of chemical partitioned to 6 compartments (air, soil, water, suspended sediment, sediment, biota) within a unit world. Level I data are basic partitioning data that allow for comparisons between chemicals and indicate the compartment(s) to which a chemical is likely to partition.

The EQC Level I is a steady state, equilibrium model that utilizes the input of basic chemical properties including molecular weight, vapor pressure, and water solubility to calculate distribution within a standardized regional environment. This model will be used to calculate distribution values for representative chemical components identified in streams in this category. A computer model, EPIWIN – version 3.02[1], will be used to calculate the properties needed to run the Level I EQC model.

B. Stream Specific Rationales

The rationales for the test plan strategy specific to each stream in the C5 Non-Cyclics category are presented below:

1. Pyrolysis C5s

This stream will be tested in a complete SIDS battery of tests (except for acute toxicity) to assess the toxicity of streams with a mid-range (approximately 14-20%) isoprene content in addition to significant amounts of other C5 dienes, 2-methyl-2-butene, dicyclopentadiene, and most of the other C5s that are present in the other streams in the C5 Non-Cyclics Category.

2. Hydrotreated C5s

This stream will be tested in a complete SIDS battery of tests (except for acute toxicity) to assess the toxicity of streams with a low (approximately 2%) isoprene content; small amounts of other C5 dienes; no dicyclopentadiene; substantial concentrations of pentenes, pentanes, cyclopentene; and approximately 11% 2-methyl-2-butene.

3. Pentenes

This stream is similar to the Hydrotreated C5s stream except that it contains approximately 4% 2-methyl-2-butene. The toxicity profile of this stream is expected to be the same as that of the Hydrotreated C5s stream. No testing is proposed for this stream at this time.

4. Piperylene Concentrate

This stream is similar to the Hydrotreated C5 stream except for the presence of 2-methyl-2-butene (typically 5-15%) and a large amount of 1,3-pentadiene (typically 30-60%). The toxicity of the Piperylene Concentrate stream can be characterized by data for 2-methyl-2-butene, 1,3-pentadiene, and the Hydrotreated C5s stream. 1,3-Pentadiene is an OECD SIDS material and all SIDS endpoints have been adequately addressed. The SIDS Initial Assessment Report (SIAR) indicates that 1,3-pentadiene (cis and trans combined) is of low concern for further testing, and proposes no additional testing. Testing of 2-methyl-2-butene and the Hydrotreated C5s stream is proposed in this Test Plan.

5. Isoprene Concentrate

This stream is similar to the Pyrolysis C5s stream except that this stream has a higher concentration of isoprene. Existing data for high purity isoprene and data from testing of the Pyrolysis C5s stream, which has approximately 14-20% isoprene, can be used to characterize the toxicity of Isoprene Concentrate. No additional testing is proposed for this stream at this time.

6. Isoprene-Piperylene Concentrate

This stream is similar to the Pyrolysis C5s stream. Data from testing of the Pyrolysis C5s stream, which has approximately 14-20% isoprene, can be used to characterize the toxicity of Isoprene-Piperylene Concentrate. No additional testing is proposed for this stream at this time.

7. Isoprene, High Purity

Isoprene has been extensively tested and all HPV endpoints except for aquatic toxicity have been adequately addressed. A significant amount of additional toxicology data is also available. An algal toxicity test is proposed for this stream. In addition, structural activity relationships will be used to predict toxicity to fish and Daphnia. Isoprene is sponsored through the ICCA HPV Program.

8. Isoprene Purification Byproduct

This stream is predominantly (50-70%) isopentane, which is being addressed by the American Chemistry Council Hydrocarbon Solvents Panel, but also has a typical isoprene content of 1-12%. The non-genetic endpoints can be addressed by the data for isopentane. The data for isoprene, Pyrolysis C5s and Hydrotreated C5s streams can be used to characterize the genetic toxicity of this stream. No additional testing is proposed for this stream, at this time.

9. 2-Methyl-2-Butene

This stream is typically 93% 2-methyl-2-butene and 6.7% 2-methyl-1-butene. It will be tested in a complete battery of tests except for genetic toxicity tests (which are available). 2-Methyl-2-butene is sold as a high-purity material and is also a component in the Pyrolysis C5s, Hydrotreated C5s, Pentenes, Isoprene Concentrate, Piperylene Concentrate, and Isoprene-Piperylene Concentrate streams. 2-Methyl-2-butene is sponsored through the ICCA HPV Program.

10. Metathesis Byproduct

This stream contains approximately 3% 2-butene, 44% pentenes, and 51% hexenes and is predicted to have a toxicology profile similar to that of the 2% isoprene stream. The toxicity of the Metathesis Byproduct stream can be adequately characterized by read-across from data for 1-hexene, which is an OECD SIDS material, and from a C6-C8 internal olefin (or similar) stream which will be tested by the American Chemistry Council Higher Olefins Panel and from results for the hydrotreated C5s stream, if test results indicate that the presence of 2% isoprene does not cause genetic effects.

11. Neohexene

This stream is typically 97% neohexene (3,3-dimethyl-1-butene) and 3% related hydrocarbons. Based on existing acute and genetic toxicity data, neohexene is expected to have a toxicity profile similar to that

of many of the C5 and C6 alkenes present in the streams in this category. The toxicity of neohexene is also expected to be similar to that of the low isoprene stream, Hydrotreated C5s, for which testing is proposed in this Test Plan. Existing data for neohexene and similar alkenes and the data from the proposed testing of the Hydrotreated C5s stream are expected to be adequate to characterize the toxicity of this stream.

IV. TEST PLAN SUMMARY

The following testing, modeling, and technical discussions will be developed for the C5 Non-Cyclics category (Table 3):

- Conduct one test battery for all SIDS human health endpoints (except acute toxicity) on a stream containing approximately 14-20% isoprene, Pyrolysis C5s (exact composition to be determined at the time of testing). The following studies will be conducted: An Ames test (OECD Guideline 471), a mouse inhalation micronucleus test (OECD Guideline 474), and a rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422).

- Conduct one test battery for all SIDS human health endpoints (except acute toxicity) on a stream containing approximately 2% isoprene, Hydrotreated C5s (exact composition to be determined at the time of testing). The following studies will be conducted: An Ames test (OECD Guideline 471), a mouse inhalation micronucleus test (OECD Guideline 474), and a rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422).

- Conduct a rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422) on a high purity 2-methyl-2-butene stream (exact composition to be determined at the time of testing). 2-Methyl-2-butene is sponsored through the ICCA HPV Program.

- Compare endpoints evaluated for the Pyrolysis C5s stream (the mid-range isoprene stream with approximately 14-20% isoprene) and the Hydrotreated C5s stream (the low isoprene stream with approximately 2% isoprene) to those for high purity isoprene and the other identified data and prepare a technical discussion in terms of their representation of potential human health effects for this category.

- Compare endpoints evaluated for the 2-methyl-2-butene stream to those for the other two tested streams and the other identified data and prepare a technical discussion in terms of their representation of potential human health effects for this category.

- Conduct alga toxicity tests (OECD Guideline 201), *Daphnia* sp acute toxicity tests (OECD Guideline 202) and fish acute toxicity tests (OECD Guideline 203) with a mid-range

isoprene stream (Pyrolysis C5s), low isoprene stream (Hydrotreated C5s), and 2-methyl-2-butene.

- Conduct an alga toxicity study (OECD Guideline 201) with high purity isoprene. Isoprene is sponsored through the ICCA HPV Program. In addition, structural activity relationships will be used to predict toxicity to fish and *Daphnia*.

- Conduct biodegradation tests (OECD Guideline 301F) with mid-range (Pyrolysis C5s) and low isoprene (Hydrotreated C5s) streams and 2-methyl-2-butene.

- Prepare a technical discussion of the potential aquatic toxicity of selected chemical components comprising streams in this category using modeled data.

- Prepare a technical discussion on the potential of chemical components comprising streams in this category to photodegrade.

- Prepare a technical discussion on the potential of chemical components comprising streams in this category to hydrolyze.

- Calculate fugacity data for selected chemical components of streams in this category.

- Calculate physicochemical data as described in the EPA document titled, *The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program*.

Summaries of results will be developed once the data and analyses are available. This test plan is expected to provide adequate data to characterize the human health effects and environmental fate and effects endpoints for the category under the Program.

V. OTHER SUPPORTING DATA

The test plan is based on the expectation that the biological activity of isoprene and 2-methyl-2-butene will be responsible for any effects seen in the testing of the C5 Non-Cyclics streams. This expectation is based on a review of a substantial amount of data that is available for many of the other components of the C5 Non-Cyclics category. Existing data is indicated in Table 4.

Additional data for components of the C5 Non-Cyclics streams that will provide support for this category will be collected by other test plans within the Olefins Panel's HPV program (see Table 5), by other consortia participating in the HPV or ICCA programs, or from chemicals sponsored in the OECD SIDS program:

- 1-Butene: Data gaps will be filled under the Olefins Panel's Low Butadiene C4 HPV Test Plan.

1-Butene is sponsored through the ICCA HPV Program.

- 2-Butene: OECD SIDS
- n-Pentane: Included in American Petroleum Institute's HPV Test Plan.
- C5 Aliphatic Category: Included in the American Chemistry Council Hydrocarbon Consortium HPV Test Plan that covers n-pentane, isopentane, and cyclopentane.
- 1-Pentene and isopentene: Although not covered by the American Chemistry Council Higher Olefins Panel's HPV Test Plan, the data obtained for hexenes within that program can be used for read-across to the pentenes. In addition, data for 1-hexene collected as part of the OECD SIDS program can be used for read-across.
- Cyclopentane: Included in American Chemistry Council Hydrocarbon Solvents Panel's HPV program.
- 1,3-Pentadiene: OECD SIDS
- Dicyclopentadiene: OECD SIDS

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5. Neely, W. B. 1985. Hydrolysis. In: W. B. Neely and G. E. Blau, eds. Environmental Exposure from Chemicals. Vol I., pp. 157-173. CRC Press, Boca Raton, FL, USA.
6. Mackay, D., A. Di Guardo, S. Paterson, and C. E. Cowan. 1996. Evaluating the Environmental Fate of a Variety of Types of Chemicals Using the EQC Model. Environ. Toxicol. Chem. 15:1627-1637.

Table 1. CAS Numbers and Descriptions Associated with Streams in C5 Non-Cyclics Category

CAS Number	CAS Number Description
513-35-9	2-Butene, 2-methyl-
558-37-2	1-Butene, 3,3-dimethyl-
64742-83-2	Naphtha, petroleum, light steam-cracked
68410-97-9	Distillates, petroleum, light distillate hydrotreating process, low-boiling
68476-43-7	Hydrocarbons, C4-6, C5-rich
68476-55-1	Hydrocarbons, C5-rich
68477-35-0	Distillates, petroleum, C3-6, piperylene-rich
68514-39-6	Naphtha, petroleum, light steam-cracked, isoprene-rich
68527-11-7	Alkenes, C5
68527-19-5	Hydrocarbons, C1-4, debutanizer fraction
68603-00-9	Distillates, petroleum, thermal cracked naphtha and gas oil
68603-03-2	Distillates, petroleum, thermal cracked naphtha and gas oil, extractive
68606-29-1	Hydrocarbons, C4 and C8, butene concentrator by-product
68606-36-0	Hydrocarbons, C5-unsatd. rich, isoprene purifn. by-product
68956-55-8	Hydrocarbons, C5-unsatd.
78-79-5	1,3-Butadiene, 2-methyl-

Note: The definitions, found in the TSCA Chemical Substance Inventory, for the CAS numbers included in this group are vague with respect to composition. Therefore, it is not uncommon to find that the same CAS number is correctly used to describe different streams (compositions) or that two or more different CAS numbers are used to describe the same stream (composition)

Table 2. Typical Stream Compositions (%) for the C5 Non-Cyclics Category

Component	Pyrolysis C5s	Hydrotreated C5s	Pentenes	Piperylene Concentrate	Isoprene Concentrate	Isoprene - Piperylene Concentrate	Isoprene	Isoprene Purification Byproduct	2-Methyl-2-Butene	Metathesis Byproduct	Neohexene
Isoprene (2-Methyl-1,3-Butadiene)	9 - 25	2	2.4	0 - 6	14 - 80	20	99.7	1 - 12			
1,4-Pentadiene	1 - 6		1		0 - 4	3		1 - 10			
1,3-Pentadiene (isomer mix)	6 -23	2		31 - 60	1 - 15	14					
3-Methyl-1,2-Butadiene					3.5						
1,3-Butadiene	0 - 3							0 - 1			
1,2-Butadiene					1.2						
1,3-Cyclopentadiene	2 - 23	5		1 - 4	0 - 15	2		0 - 1			
Dicyclopentadiene	1 - 19			0 - 1	0.4						
Cyclopentene	1 - 11	15 - 20		8 - 20	0 - 10	4					
Cyclopentane	0 - 2	2		1 - 5	8 - 15	1					
Isopentene		15 - 20									
1-Pentene (Amylene)	3 - 12		13.7		0 - 10	6		3 - 11		3	
2-Pentene (isomer mix)	2 - 10		18	1 - 10	1 - 16	5		3 - 9		41	
2-Methyl-2-Butene	1 - 5	11	4	5 - 15	0 - 9	3			93		
2-Methyl-1-Butene	1 - 8		17.5		1 - 16	6			6.7		
3-Methyl-1-Butene (Isoamylene)	0 -12				0 - 1	1					
Methyl Butenes								3 - 21			
Isopentane (2-Methylbutane)	3 - 29	15 - 25	12.3		21	16		50 - 70			
n-Pentene		10 - 15									
Pentane	4 - 30	15 - 20	31.7	0 - 5	0 - 26	16		0 - 10			
3-Methyl- 1,4-Pentadiene				1.7							
3-Methyl-1-Pentene	0 - 2										

Component	Pyrolysis C5s	Hydrotreated C5s	Pentenes	Piperylene Concentrate	Isoprene Concentrate	Isoprene - Piperylene Concentrate	Isoprene	Isoprene Purification Byproduct	2-Methyl-2-Butene	Metathesis Byproduct	Neohexene
Methylpentenes				5							
2,3-Dimethy-1-Butene											1.5
C6 Hydrocarbons	2 - 4		1	1 - 5	0 - 3						
1-Hexene	0 - 3									4	
2-Hexene										15	
3-Hexene										8	
Hexenes		1		2							
Methyl-2-Pentenes										24	
2,2-Dimethylbutane (neohexane)	0 - 1			2.7							
3,3-Dimethyl-1-Butene (Neohexene)											97
2-Methylpentane		5									
Methylpentanes				16							
Hexane		1		3.3							
Benzene	0 - 1	1		0.2							
Dimers of CPD with other C4 and C5 Dienes, excluding DCPD	0 - 2										
2-Butyne (Dimethylacetylene)	0 - 2				1 - 2						
1-Butene		2									
2-Butene (isomer mix)	0 - 1				1 - 20					3	

same Note 1:The balance of these streams is expected to be other hydrocarbons that have boiling points in the range of the listed components.
Note 2: The listed ranges should not be considered absolute values. They are instead the approximate highs and lows of the reported values, and are expected to be typical limit values.
Note 3: The definitions, found in the TSCA Chemical Substance Inventory, for the CAS numbers included in this group are vague with respect to composition. Therefore, it is not uncommon to find that the same CAS number is correctly used to describe different streams (compositions) or that two or more different CAS numbers are used to describe the stream

(composition).

Table 3. Assessment Plan for C5 Non-Cyclics Category Under the Program. Robust summaries for existing studies are submitted separately.

Stream Description	Human Health Effects						Ecotoxicity			Environmental Fate				
	Acute Toxicity	Genetic Point Mut.	Genetic Chrom.	Sub-chronic	Develop-mental	Reproduc-tion	Acute Fish	Acute Invert.	Algal Toxicity	Physical Chem.	Photo-deg.	Hydro-lysis	Fugacity	Biodeg.
C5 Non-Cyclics Streams Containing Isoprene														
Isoprene (1,3-Butadiene, 3-methyl) , High Purity (Isoprene Content = 100%) *	Ö	Ö	Ö	Ö	Ö	Ö	CM	CM	T	CM	CM/TD	TD	CM	Ö
Isoprene Concentrate (Isoprene Content = 14-85%)	RA	RA	RA	RA	RA	RA	RA	RA	RA	CM	CM/TD	TD	CM	RA
Pyrolysis C5s (Isoprene Content = 14-20%)	NA	T	T	T	T	T	T	T	T	CM	CM/TD	TD	CM	T
Isoprene-Piperylene Concentrate (Isoprene Content = 20%)	RA	RA	RA	RA	RA	RA	RA	RA	RA	CM	CM/TD	TD	CM	RA
Isoprene Purification Byproduct (Isoprene Content = 1-12%)	RA	RA	RA	RA	RA	RA	RA	RA	RA	CM	CM/TD	TD	CM	RA
Piperylene Concentrate (Isoprene Content = 0-6%)	RA	RA	RA	RA	RA	RA	RA	RA	RA	CM	CM/TD	TD	CM	RA
Pentenes (Isoprene Content = 2%)	RA	RA	RA	RA	RA	RA	RA	RA	RA	CM	CM/TD	TD	CM	RA
Hydrotreated C5s (Isoprene Content = 2%)	NA	T	T	T	T	T	T	T	T	CM	CM/TD	TD	CM	T
Streams Not Containing Isoprene but Containing Other Components Found in Streams in C5 Non-Cyclics Category														
2-Methyl-2-Butene (≥ 93%)*	NA	Ö	Ö	T	T	T	T	T	T	CM	CM/TD	TD	CM	T
Metathesis Byproduct (Pentenes, Hexenes)	RA	RA	RA	RA	RA	RA	RA	RA	RA	CM	CM/TD	TD	CM	RA
Neohexene (3,3-dimethyl-1-Butene, ≥97%)	Ö	Ö	RA	RA	RA	RA	RA	RA	RA	CM	CM/TD	TD	CM	RA

Ö Adequate existing data available TD Technical discussion proposed RA Read Across (see Sec. III.B)
CM Computer Modeling proposed T Proposed Testing * Sponsored through ICCA

NA Not Applicable

Table 4. Existing Data for Components Other Than Isoprene and 2-Methyl-2-Butene

(Robust summaries for these studies will not be submitted with the Test Plan; some studies have not been reviewed for adequacy)

		Human Health Effects						Ecotoxicity			Environmental Fate				
CAS Number	**Chemical Name**	**Acute Oral**	**Genetic Point Mutation**	**Genetic Chrom. Aberr.**	**Sub-chronic**	**Develop-mental**	**Repro-duction**	**Acute Fish**	**Acute Invert.**	**Algal Toxicity**	**Physical Chem.**	**Photo-degra-dation**	**Hydro-lysis**	**Fugacity**	**Biodegra-dation**
78-78-4	Isopentane (2-methyl-butane)	√	√		√										√
590-18-1	Cis-2-butene	√													
109-66-0	Pentane	√	√	√	√	√		√	√	√					√
107-83-5	2-Methylpentane (isohexane)				√										
646-04-8	Trans-2-pentene				√										
142-29-0	Cyclopentene	√													
287-92-3	Cyclopentane	√			√										
504-60-9	1,3-Pentadiene (SIDS SIAR complete)	√	√	√	√	√	√	√	√	√					√
542-92-7	Cyclopentadiene	√			√										
26760-64-5	2-methyl-1-butene			√											
77-73-6	Dicyclopentadiene	√	√	√	√	√	√	√	√	√					√
110-54-3	Hexane	√	√	√	√	√	√	√	√						

Table 5. American Chemistry Council Olefins Panel Sponsored HPV Test Categories

Category Number	Category Description
1	Crude Butadiene C4
2	Low Butadiene C4
3	C5 Non-Cyclics
4	Propylene Streams (C3) - Propylene sponsored through ICCA
5	High Benzene Naphthas
6	Low Benzene Naphthas
7	Resin Oil - High Dicyclopentadiene
8	Resin Oil - Low Dicyclopentadiene
9	Cyclodiene Concentrates
10	Fuel Oils

Appendix I

ETHYLENE PROCESS DESCRIPTION

A. The Ethylene Process

1. Steam Cracking

Steam cracking is the predominant process used to produce ethylene. Various hydrocarbon feedstocks are used in the production of ethylene by steam cracking, including ethane, propane, butane, and liquid petroleum fractions such as condensate, naphtha, and gas oils. The feedstocks are normally saturated hydrocarbons but may contain minor amounts of unsaturates. These feedstocks are charged to the coils of a cracking furnace. Heat is transferred through the metal walls of the coils to the feedstock from hot flue gas, which is generated by combustion of fuels in the furnace firebox. The outlet of the cracking coil is usually maintained at relatively low pressure in order to obtain good yields to the desired products. Steam is also added to the coil and serves as a diluent to improve yields and to control coke formation. This step of the ethylene process is commonly referred to as "steam cracking" or simply "cracking" and the furnaces are frequently referred to as "crackers".

Subjecting the feedstocks to high temperatures results in the partial conversion of the feedstock to olefins. In the simplest example, feedstock ethane is partially converted to ethylene and hydrogen. Similarly, propane, butane, or the liquid feedstocks are also converted to ethylene. While the predominant products produced are ethylene and propylene, a wide range of additional products are also formed. These products range from methane (C1) through fuel oil (C12 and higher) and include other olefins, diolefins, aromatics and saturates (naphthenes and paraffins).

2. Refinery Gas Separation

Ethylene and propylene are also produced by separation of these olefins from refinery gas streams, such as from the light ends product of a catalytic cracking process or from coker offgas. This separation is similar to that used in steam crackers, and in some cases both refinery gas streams and steam cracking furnace effluents are combined and processed in a single finishing section. These refinery gas streams differ from cracked gas in that the refinery streams have a much narrower carbon number distribution, predominantly C2 and/or C3. Thus the finishing of these refinery gas streams yields primary ethylene and ethane, and/or propylene and propane.

B. Products of the Ethylene Process

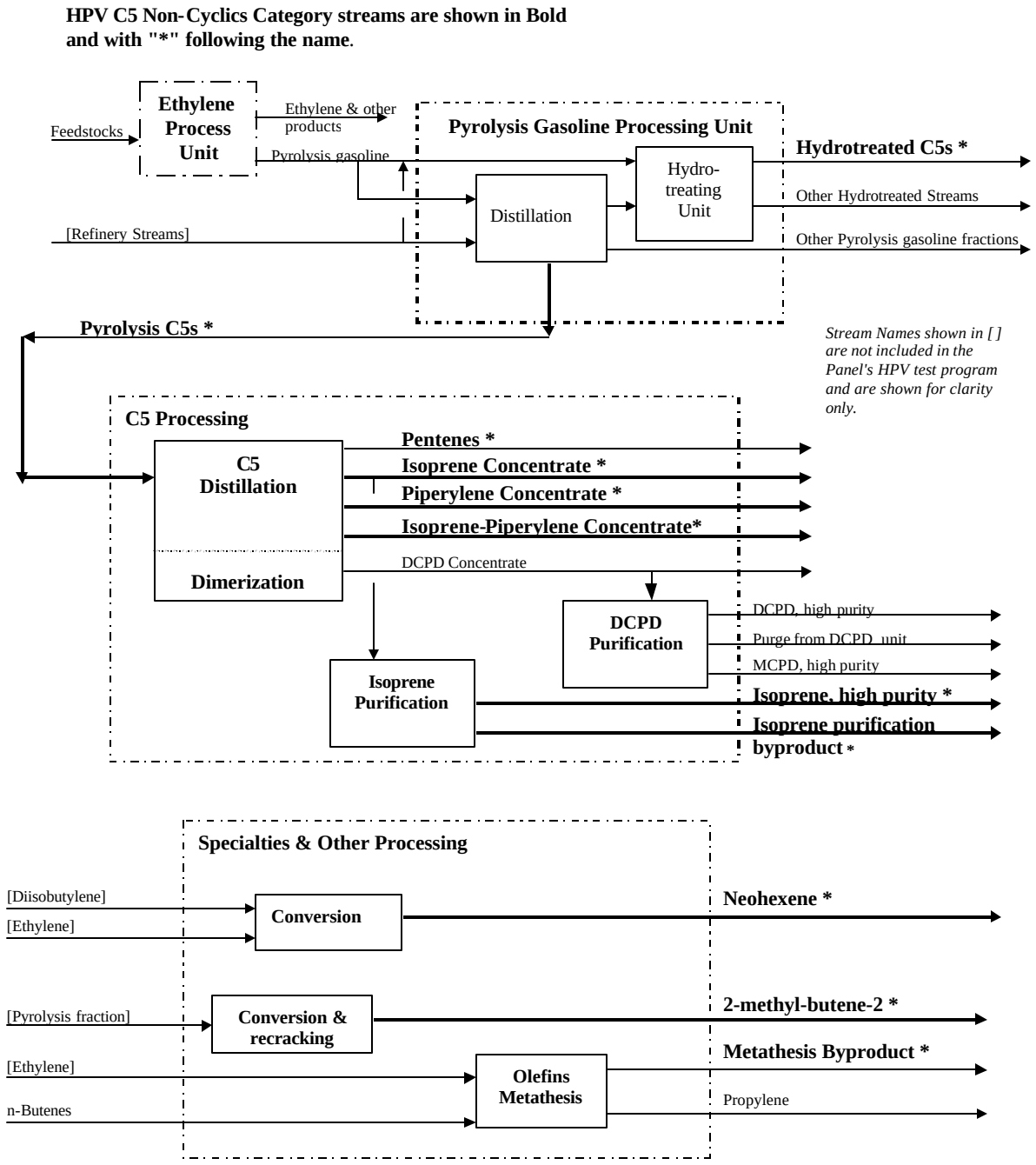
The intermediate stream that exits the cracking furnaces (i.e., the furnace effluent) is forwarded to the finishing section of the ethylene plant. The furnace effluent is commonly referred to as "cracked gas" and consists of a mixture of hydrogen, methane, and various hydrocarbon compounds with two

or more carbon atoms per molecule (C2+). The relative amount of each component in the cracked gas varies depending on what feedstocks are cracked and cracking process variables. Cracked gas may also contain relatively small concentrations of organic sulfur compounds that were present as impurities in the feedstock or were added to the feedstock to control coke formation. The cracked gas stream is cooled, compressed and then separated into the individual streams of the ethylene process. These streams can be sold commercially and/or put into further steps of the process to produce additional materials. In some ethylene processes, a liquid fuel oil product is produced when the cracked gas is initially cooled. The ethylene process is a closed process and the products are contained in pressure systems.

The final products of the ethylene process include hydrogen, methane (frequently used as fuel), and the high purity products ethylene and propylene. Other products of the ethylene process are typically mixed streams that are isolated by distillation according to boiling point ranges. It is a subset of these mixed streams that make up the constituents of the C5 Non-Cyclics category.

The chemical process operations that are associated with the process streams in the C5 Noncyclics category are shown in Figure 1.

Figure 1. Chemical process operations associated with process streams in the C5 Noncyclics category.



AR 201-12801B

Robust **Summary - Group** 3: **C5** Non-Cyclics

Acute Toxicity

Test Substance	Isoprene, CAS# 78-79-S
Method	
Method/guideline followed	Other.
Type (test type)	Acute inhalation -LC_{50}
GLP	Pre-GLP
Year	1969
Species/Strain	Rat and mouse (strains not specified)
Sex	Not specified
No. of animals per sex per dose	Not specified
Vehicle	Not applicable
Route of administration	Inhalation (vapor)
Test Conditions	Age, number, and sex of test animals not specified. Number of groups and exposure concentrations not specified. Dynamic flow exposure system; no description of exposure chambers or conditions. Rats exposed four hours; mice exposed two hours. No post-exposure observation period - mortality study only. Exposure concentrations "controlled" by gas chromatography. LC50 calculation by probit-analysis according to Litchfield and Wilcoxon.
Results	
LC_{50} with confidence limits.	Rat LC_{50} (4 hr) = 180 mg/L (64,620 ppm); confidence limits 130-181 mg/L ($p \leq 0.05$). Mouse LC_{50} (2 hr) = 157 mg/L (56,363 ppm); confidence limits 129-252 mg/L ($p \leq 0.05$). No clinical observations or necropsy findings reported. Objective of study was to determine hydrocarbon concentrations in various tissues at lethal exposure concentrations.
Conclusions (study author)	LC50 value reported to be 180 mg/L (64,620 ppm) in rats, 157 mg/L (56,363 ppm) in mice.
Data Quality	
Reliability	4 - Not assignable. Lethality study only; insufficient experimental detail to assess quality.
References	Shugaev, B.B. (1969) Concentrations of Hydrocarbons in Tissues as a Measure of Toxicity. Arch. Environ. Health 18:878-882.
Other	
Last changed	21-Aug-00 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vitro

Test Substance	Isoprene, CAS# 78-79-5
Test substance	Purity >99%.
Method	
Method/guideline followed	OECD 47 1
Type	Ames Salmonella/bacterial reverse mutation test (pre-incubation assay).
System of testing	Bacterial.
GLP	Yes.
Year	1986
Species/Strain	*Salmonella* / TA98, TA100, TA1535, TA1537.
Metabolic activation	With and without.
Species and cell type	Rat and hamster liver S9 fraction.
Quantity	0.5 ml/plate.
Induced or not induced	Arochlor 1254-induced (500 mg/kg for 5 days).
Concentrations tested	0, 100, 333, 1000, 3333, 10000 ug/plate.
Statistical Methods	A positive response was defined as a reproducible, dose-related increase in revertant colonies in any **one** strain/activation combination. There was no minimum percentage or fold increase required for the chemical to be judged positive or weakly positive.
Test Conditions	The preincubation modification of the Salmonella/mammalian microsome assay was used to test isoprene in five different ***Salmonella*** strains in the presence and absence of rat and hamster liver S-9. Five dose levels were tested , with three plates per dose level. The high dose was limited by toxicity to 10,000 ug/plate. Concurrent positive controls were also tested with and without metabolic activation. The assay was repeated less than one week after completion of the initial test.
Results	
Genotoxic effects	Negative. Isoprene was not mutagenic in any of the five strains of ***Salmonella*** tested in the presence or absence of Aroclor-induced rat or hamster liver S9.
Conclusions (contractor)	Isoprene was not mutagenic in the Ames Salmonella mutagenicity test.
Data Quality *Reliabilities*	1 - Reliable without restrictions. Evaluated as part of a NTP-sponsored interlaboratory study of 270 chemicals.
Reference	Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., and Zeiger, E. (1986) Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environ. Mutagen. 8 (Suppl. 7): l-l 19.
Other	
Last changed	20-Aug-00 Robust summary prepared by a contractor to the Panel

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vitro

Test Substance	Isoprene, CAS# 78-79-5
Test substance	Purity >99%.
Method	
Method/guideline followed	OECD 479
Type	*In vitro* Sister Chromatid Exchange (SCE) Assay in Mammalian Cells
System of testing	Chinese hamster ovary (CHO) cells.
GLP	Yes.
Year	1987.
Metabolic activation	Aroclor 1254-induced Sprague-Dawley rat liver S9.
Concentrations tested	50, 160, 500, 1600 ug/ml (without S9), or 160,500, 1600, 5000 ug/ml (with S9).
Control groups and treatment	Solvent controls: dimethylsulfoxide; positive controls: Mitomycin-C (without S9), cyclophosphamide (with S9).
Statistical Methods	Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points. A frequency 20% above the solvent control group was considered positive. Positive trend tests ($p \leq 0.05$) in the absence of a significant difference at any one dose were considered equivocal.
Test Conditions	Isoprene was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCE) both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and four doses of isoprene. A single flask per dose was used. All **slides** were scored blind and those from a single test were read by the same person. Fifty 2nd-division metaphase cells were scored for frequency of SCEs/cell from each dose level.
Results	
Genotoxic effects	Negative. No increases in SCEs were noted in cultured CHO cells treated with isoprene, with or without S9.
Conclusions (contractor)	Isoprene did not induce sister chromatid exchanges *in vitro* in cultures of Chinese hamster ovary cells.
Data Quality	
Reliabilities	1 - Reliable without restrictions. Evaluated as part of an NTP-sponsored study of 108 chemicals.
Reference	Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environ Mol. Mutagen lO:l-175.
Other	
Last changed	2 1 -Aug-00 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vitro

Test Substance	Isoprene, CAS# 78-79-5
Test substance	Purity >99%.
Method	
Method/guideline followed	OECD 473
Type	*In vitro* Mammalian Chromosomal Aberration Test.
System of testing	Chinese hamster ovary (CHO) cells.
GLP	Yes.
Year	1987.
Metabolic activation	Aroclor 1254-induced Sprague-Dawley rat liver S9.
Concentrations tested	1600, 3000, 5000 ug/ml.
Control groups and treatment	Solvent control: dimethylsulfoxide; positive controls: Mitomycin-C (without S9), cyclophosphamide (with S9).
Statistical Methods	Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points. A statistically significant ($p \leq 0.05$) difference for one point and a significant trend ($p \leq 0.015$) was considered positive. Positive trend tests ($p \leq 0.05$) in the absence of a significant difference at any one dose were considered equivocal.
Test Conditions	Isoprene **was tested** in cultured Chinese hamster ovary (CHO) cells for induction of chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and three doses of isoprene. A single flask per dose was used. All slides were scored blind and those from a single test were read by the same person. Two hundred 1st-division metaphase cells were scored for chromosomal aberrations at each dose level.
Results	
Genotoxic effects	Negative. No increases in chromosomal aberrations were noted in cultured CHO cells treated with isoprene, with or without S9.
Conclusions	Isoprene did not induce chromosomal aberrations *in vitro* in cultures of Chinese hamster
(contractor)	ovary cells.
Data Quality	1 - Reliable without restrictions. Evaluated as part of a NTP-sponsored study of 108
Reliabilities	chemicals.
Reference	Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environ Mol. Mutagen 10:1-175.
Other	
Last changed	**2** 1 -Aug-00 Robust summary prepared by a contractor to the Panel.

Genetic Toxicity -- In Vivo

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >98%.
Method	
Method/guideline followed	Other.
Type	*Zn vivo* Sister Chromatid Exchange (mouse bone marrow cytogenetics study) .
GLP	Yes.
Year	1988.
Species	Mouse
Strain	B6C3F1.
Sex	15 male/group.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0,438, 1750, 7000 ppm.
Exposure period	6 hours/day for 12 days.
Statistical methods	The frequencies of sister chromatid exchanges (SCEs) were analyzed for increasing trend by the one-tailed Cochran-Armitrage trend test ($p<0.05$). For data exhibiting a significant trend, pairwise comparisons between each exposure group and the concurrent control were performed using either the one-tailed or two-tailed t-test.
Test Conditions	Fifteen male B6C3F1 mice (approximately 6-7 weeks old) per group were exposed for 12 days, 6 h/day to 0,438, 1750, or 7000 ppm of isoprene by inhalation. The exposure regimen was 3 exposure days, 2 days off, 5 exposure days, 2 days off, then 4 exposure days. Exposure concentrations were monitored by gas chromatography. The animals were implanted with a BrdU tablet 1 hour before the 12th exposure. Two hours before sacrifice on the following day, the animals received an intraperitoneal injection of colchicine. For analysis of SCE, 5 mice per exposure group were killed 24 hours after BrdU implantation. Bone marrow was removed, fixed onto slides, and stained using differential chromatid staining. Twenty-five second-division metaphase cells were scored for SCEs from 4 mice/group. Additionally, 100 randomly selected metaphase cells per slide were scored for replication history to provide data on cell generation time, a measure of cell proliferation kinetics. The percentage of cell in metaphase among 1000 cells/sample was used to calculate the mitotic index.
Results	
Genotoxic effects	Positive.
NOAEL (NOEL)	<438 ppm.
LOAEL (LOEL)	438 ppm.
	Exposure to isoprene for 6 h/day at 0,438, 1750, or 7000 ppm for 12 days induced a significant increase in the frequency of SCEs in bone marrow cells, The increased SCE responses in the exposed groups were not significantly different from each other. Analysis of average generation time and mitotic index data indicated no change in the percentage of bone marrow cells engaged in division but a significant lengthening of the cell cycle duration of proliferating cells in the 7000 ppm group. There were no significant clinical signs or mortality throughout the study.
Conclusions (study authors)	Isoprene was found to be genotoxic and cytotoxic to mouse bone marrow *in vivo* - inducing SCE, inhibiting cellular proliferation, and suppressing the rate of erythropoiesis. The lack of significant difference in SCEs among the three exposed groups suggests a saturation of the metabolic capacity of male mice to form reactive species.
Data Quality	
Reliabilities	1 - Reliable without restrictions. NTP-sponsored study.
References	Tice, R.R., Boucher, R., Luke, C.A., Paquette, D.E., Melnick, R.L., and Shelby, M.D. (1988). Chloroprene and isoprene: cytogenetic studies in mice. Mutagenesis 3(2): 14 1-146.
Other	
Last changed	26-Apr-00
	Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vivo

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >98%.
Method	
Method/guideline followed	OECD 475
Type	Mammalian Bone Marrow Chromosomal Aberration Test.
GLP	Yes.
Year	1988.
Species	Mouse
Strain	B6C3F1
Sex	15 male/group.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0,438, 1750, 7000 ppm.
Exposure period	6 hours/day for 12 days.
Statistical methods	The frequencies of chromosomal aberrations (Abs) were analyzed for increasing trend by the one-tailed Cochran-Armitrage trend test ($p<0.05$). For data exhibiting a significant trend, pairwise comparisons between each exposure group and the concurrent control were performed using either the one-tailed or two-tailed t-test.
Test Conditions	Fifteen male B6C3F 1 mice (approximately 6-7 weeks old) per group were exposed for 12 days, 6 h/day to 0, 438, 1750, or 7000 ppm of isoprene by inhalation. The exposure regimen was 3 exposure days, 2 days off, 5 exposure days, 2 days off, then 4 exposure days. Exposure concentrations were monitored by gas chromatography. The animals were implanted with a BrdU tablet 1 hour before the 12th exposure. Two hours before sacrifice on the following day, the animals received an intraperitoneal injection of colchicine. For analysis of Abs, 10 mice per exposure group were killed 17-20 hours after BrdU implantation. Bone marrow was removed, fixed onto slides, and stained using differential chromatid staining. Fifty first-division metaphase cells were scored for Abs from 8 mice/group.
Results	
Genotoxic effects	Negative.
NOAEL (NOEL)	7000 ppm
LOAEL (LOEL)	>7000 ppm
	Exposure to isoprene for 6 h/day at 0,438, 1750, or 7000 ppm for 12 days did not induce a statistically significant increase in the frequency of chromosomal aberrations (Abs) in bone marrow cells. The incidence of bone marrow cells with chromosomal aberrations (Abs) was slightly elevated in the exposed groups compared to the control, but these increases were not statistically significant.
Conclusions (study authors)	The incidence of bone marrow cells with chromosomal aberrations in male mice treated with isoprene for 12 days was not significantly increased.
Data Quality	
Reliabilities	1 - Reliable without restrictions. NTP-sponsored study.
References	Tice, R.R., Boucher, R., Luke, C.A., Paquette, D.E., Melnick, R.L., and Shelby, M.D. (1988). Chloroprene and isoprene: cytogenetic studies in mice. Mutagenesis 3(2): 141-146.
Other	
Last changed	21-Aug-00
	Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vivo

Test Substance	Isoprene, CAS# 78-79-S
Remarks	Purity >98%.
Method	
Method/guideline followed	OECD 474
Type	Mammalian Erythrocyte Micronucleus Test
GLP	Yes.
Year	1988.
Species	Mouse
Strain	B6C3F1
Sex	15 male/group.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0,438, 1750, 7000 ppm.
Exposure period	6 hours/day for 12 days.
Statistical methods	The number of micronucleated erythrocytes (MN) were summed across animals within each group and analyzed for increasing trend by a one-tailed trend test ($p<0.05$). For data exhibiting a significant trend, pairwise comparisons between each exposure group and the concurrent control were performed using a one-tailed Pearson Chi square test to determine the minimal effective dose.
Test Conditions	Approximately 24 hours following the last exposure peripheral blood samples were obtained from each animal by tail snip, immediately air-dried and fixed with methanol. One thousand polychromatic erythrocytes (PCEs) and 1000 normochromatic erythrocytes (NCEs) were scored per animal for frequency of micronucleated erythrocytes (MN). The percentage of PCEs in 1000 erythrocytes was also determined as a measure of isoprene-induced toxicity.
Results	
Genotoxic effects	Positive.
NOAEL (NOEL)	<438 ppm.
LOAEL (LOEL)	438 ppm.
	Exposure to isoprene for 6 h/day at 0,438, 1750, or 7000 ppm for 12 days induced a statistically significant increase in the frequency of micronucleated PCEs and NCEs in male mice at all exposure levels tested. The responses at the 1750 and 7000 ppm levels were greater than the 438 ppm level, but not different from each other. There were no significant clinical signs or mortality throughout the study.
Conclusions (study authors)	Isoprene was found to be genotoxic to mouse bone marrow *in vivo* by inducing increased MN in the peripheral blood of male mice.
Data Quality	
Reliabilities	1 - Reliable without restrictions. NTP-sponsored study.
References	Tice, R.R., Boucher, R., Luke, C.A., Paquette, D.E., Melnick, R.L., and Shelby, M.D. (1988). Chloroprene and isoprene: cytogenetic studies in mice. Mutagenesis 3(2): 14 1-146.
Other	
Last changed	2 l-Aug-00 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vivo

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >99.7%.
Method	
Method/guideline followed	Other.
Type	Rat Lung Fibroblast Micronucleus Test
GLP	Yes.
Year	1997.
Species	Rat
Strain	Fischer 344
Sex	10 male and 10 female/group.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0, 220, 700, or 7000 ppm.
Exposure period	6 hours/day, 5 days/week, for 4 weeks.
Statistical methods	Means, standard deviations, and standard error of the mean for the number of mononucleated cells/1000 binucleated cells and micronuclei/1000 binucleated cells were calculated. A two-way analysis of variance was used to analyze the measurements. Intergroup differences were delineated by Tukey's studentized range test.
Test Conditions	This study was performed in conjunction with a two-year carcinogenicity study. Groups of 10 male and 10 female rats (approximately 6-7 weeks old) per group were exposed for 4 weeks (17-19 total exposures) to 0, 220, 700, or 7000 ppm of isoprene by inhalation. The rats received at least two consecutive days of exposure prior to sacrifice and lung cell isolation. Lung fibroblasts were isolated and cultured in single-chamber slides for 72 hours. The slides were fixed and stained (acridine orange), and 1000 binucleated cells on each of two slides per animal were scored. The number of mononucleated cells and micronuclei were recorded following a standard scoring criteria.
Results	
Genotoxic effects	Negative. There were no statistically significant differences between the male or female exposed and control groups for micronucleated rat lung fibroblasts. There were no significant clinical signs or mortality during the exposure period.
Conclusions (study authors)	No significant increase in the frequency of micronucleated lung fibroblasts was observed in male and female rats exposed to isoprene for 4 weeks.
Data Quality *Reliabilities*	2 - Reliable with restrictions. Non-standard method, but comparable to guideline study. Conducted as part of NTP two-year carcinogenicity study.
References	National Toxicology Program (1997). Toxicology and Carcinogenesis Studies of Isoprene (CAS No. 78-79-5) in F344/N Rats (Inhalation Studies). Report No. TR-486.
Other	
Last changed	21-Aug-00 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Repeated Dose Toxicity

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >99%.
Method	
Method/guideline followed	Other.
Test type	2-week inhalation study.
GLP	Yes.
Year	1990.
Species	Rat and mouse.
Strain	F344 rats and B6C3F 1 mice.
Route of administration	Inhalation (vapor).
Duration of test	2 weeks.
Doses/concentration levels	0, 438, 875, 1750, 3500, or 7000 ppm.
Sex	20 male, 20 female per group.
Exposure period	6 hours/day.
Frequency of treatment	5 days/week.
Control group and treatment	20 male, 20 female, air-only exposed.
Post exposure observation period	Not applicable.
Statistical methods	Group mean body weights, organ weights, organ weight ratios, and clinical pathology results compared to controls by Dunnett's t-test.
Test Conditions	Groups of 20 animals /sex /group/species (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for two weeks (10 exposures). Ten animals/sex/group/species were used for clinical pathology evaluations after 4 (rats) or 5 (mice) exposures. The remaining ten animals per group were used for histopathology at the end of the study. Body weights and clinical observations were recorded weekly. Necropsies were performed and major tissues/organs preserved. Histopathologic examinations were performed on the control and high exposure animals (7000 ppm), and lower dose groups until an apparent no-observed -effect level was found.
Results	
NOAEL (NOEL)	7000 ppm rats, not determined for mice.
LOAEL (LOEL)	>7000 ppm rats, 438 ppm mice.
	In rats, there were no exposure-related effects observed for survival, body weight gain, clinical signs, hematologic or clinical chemistry parameters, organ weights, or the incidence of gross or microscopic lesions. In mice, there were no effects on survival; the mean body weight gain of males in the 7,000 ppm group was less than that of the controls. In mice, exposure to isoprene caused decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts in all exposed groups. Organ weight changes were observed in both male and female mice; increased liver weights and decreased thymus, spleen, and testis weights were observed in all exposed groups. Microscopic lesions observed in the exposed mice included atrophy of the testis and thymus, cytoplasmic vacuolization of the liver, olfactory epithelial degeneration in the nasal cavity, and epithelial hyperplasia in the forestomach.
Conclusions (contractor)	Isoprene exposures over 2 weeks induced changes in hematological parameters, body and organ weights, and microscopic appearances in certain tissues at levels as low as 438 ppm in the mouse whereas no changes were noted in measured parameters in the rat at exposures up to 7000 ppm. The lack of any observable toxicological effects in F344 rats exposed to isoprene for two weeks provides evidence for a species difference between rats and mice in susceptibility to isoprene.
Data Quality	
Reliabilities	1 - Reliable without restrictions. Comparable to guideline study (OECD 412).
References	Melnick, R.L., Roycroft, J.H., Chou, B.J., Ragan, H.A., and Miller, R.A. (1990). Inhalation toxicology of isoprene in F344 and B6C3F 1 mice following two-week exposures. Environ. Health Perspect. 86:93-98.
Other	
Last changed	2 1 -Aug-00 Robust summary prepared by a contractor to the Panel.

Repeated Dose Toxicity

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >99%.
Method	
Method/guideline followed	Other.
Test type	13-week inhalation study.
GLP	Yes.
Year	1994.
Species	Rat and mouse.
Strain	F344 rats and B6C3F1 mice.
Route of administration	Inhalation (vapor).
Duration of test	13 weeks.
Doses/concentration levels	0, 70, 220, 700, 2200, or 7000 ppm.
Sex	10 male, 10 female per group.
Exposure period	6 hours/day.
Frequency of treatment	5 days/week.
Control group and treatment	10 male, 10 female, air-only exposed.
Post exposure observation period	Not applicable.
Statistical methods	Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods.
Test Conditions	Groups of 10 animals /sex /group/species (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for thirteen weeks. Body weights and clinical observations were recorded weekly. Blood samples were collected for clinical pathology evaluations on days 4, 24, and at the end of the study. Urine samples were collected from rats during week 12. After thirteen weeks of exposures, all rats and mice were sacrificed and evaluated histopathologically. Organ weights were recorded.
Results	
NOAEL (NOEL)	7000 ppm rats, 220 ppm mice.
LOAEL (LOEL)	>7000 ppm rats, 700 ppm mice.
	In rats, there were no exposure-related effects observed for survival, body weight gain, clinical signs of toxicity, hematology or clinical chemistry parameters, urinalysis, organ weights, or the incidence of gross or microscopic lesions. In mice, there were no effects on survival, body weight gain, or clinical signs of toxicity. The male and female mice exposed to 700 ppm and higher showed hematologic effects indicative of a nonresponsive, macrocytic anemia at day 24 and after thirteen weeks. The incidences of focal epithelial hyperplasia of the forestomach were 0, 0, 0, 9, 8, 9 in the males, and 0, 0, 0, 10, 9, 10 in the females at 0, 70, 220, 700, 2200, and 7000 ppm (n=10). Degeneration of the olfactory epithelium and cytoplasmic degeneration of the liver were observed in 10/10 male mice at 7000 ppm. The male mice exposed to 7000 ppm exhibited testicular weights reduced 35% compared to the controls.
Conclusions (contractor)	No toxicological effects were evident in rats exposed up to 7000 ppm isoprene for 13 weeks. In mice, hematological and histopathological changes were observed at exposures of 700 ppm and higher. This 13-week subchronic inhalation study, conducted as part of a 26-week carcinogenicity study, confirmed the species difference between rats and mice in susceptibility to isoprene.
Data Quality	
Reliabilities	1 ▪ Reliable without restrictions. Comparable to guideline study (OECD 4 13).
References	Melnick RL; Sills RC; Roycroft JH; Chou BJ; Ragan HA; Miller RA (1994). Isoprene, an endogenous hydrocarbon and industrial chemical, induces multiple organ neoplasia in rodents after 26 weeks of inhalation exposure. Cancer Res. 54:5333-5339.
Other	
Last changed	21-Aug-00
	Robust summary prepared by a contractor to the Panel.

Repeated Dose Toxicity

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >99%.
Method	
Method/guideline followed	Other.
Test type	26-week inhalation study.
GLP	Yes.
Year	1994.
Species	Rat and mouse.
Strain	F344 rats and B6C3F1 mice.
Route of administration	Inhalation (vapor).
Duration of test	26 weeks,
Doses/concentration levels	0, 70, 220, 700, 2200, or 7000 ppm.
Sex	40 male rats and 40 male mice per group.
Exposure period	6 hours/day.
Frequency of treatment	5 days/week.
Control group and treatment	40 male rats and 40 male mice, air-only exposed.
Post exposure observation period	26-week post-exposure **recovery** period.
Statistical methods	Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods.
Test Conditions	Groups of 40 animals /sex /group/species (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for 26 weeks. At the end of the 26-week exposure period, 10 rats and 10 mice/group were sacrificed and evaluated. The remaining animals were allowed to recovery for an additional 26 weeks without exposure at which time they were also sacrificed and evaluated. Body weights and clinical observations were recorded weekly throughout the study. Blood samples were collected for clinical pathology evaluations after 26 weeks exposure. Tissues preserved at the 26 and 52 week sacrifices were examined microscopically. Organ weights were recorded at both intervals. Twenty mice/group were evaluated for forelimb and hindlimb grip strength after 26 weeks exposure; 10 mice/group were also evaluated at 2 days, l-, 3-, and 6-months post-exposure.
Results	
NOAEL (NOEL)	>7000 ppm rats, 70 ppm mice.
LOAEL (LOEL)	7000 ppm rats, 700 ppm mice.
	The only effect observed in the male rats after 26 weeks of exposure was interstitial cell hyperplasia of the testis (10/10) in the 7000 ppm group; following the 26-week recovery period the only effect in rats was a marginal increase in benign testicular interstitial cell tumors (9/30 at 7000 ppm). Survival of mice was reduced in the 7000 ppm group; early deaths were attributed to various neoplastic lesions and moribund sacrifices due to hindlimb paralysis. In male mice, incidences of malignant neoplastic lesions in the liver, lung, forestomach, and harderian gland were significantly increased following the 26-week exposure and 26-week recovery periods at 700 ppm and higher exposures. Non-neoplastic lesions were observed in male mice exposed to isoprene and included spinal cord degeneration (≥70 ppm) and degeneration of the olfactory epithelium (≥220 ppm). Slight increases in testicular atrophy, epithelial hyperplasia of the forestomach, partial hindlimb paralysis and a nonresponsive macrocytic anemia were also seen in male mice.
Conclusions (study authors)	Isoprene was carcinogenic to the liver, lung, forestomach, and harderian gland of male mice after 26 weeks exposure and 26 weeks recovery. In contast, the only effect observed in male rats was a marginally increased incidence of benign testicular adenomas at the highest exposure level (7000 ppm).
Quality	
Reliabilities	2 - Reliable with restrictions. Comparable to guideline studies. This study involved exposures of male rats and male mice to isoprene for 6 months, therefore provided

	additional data on repeated dose toxicity and carcinogenicity.
References	Melnick RL; Sills RC; Roycroft JH; Chou BJ; Ragan HA; Miller RA (1994). Isoprene, an endogenous hydrocarbon and industrial chemical, induces multiple organ neoplasia in rodents after 26 weeks of inhalation exposure. Cancer Res. 54:5333-5339.
Other	
Last changed	**2** 1-Aug-00 Robust summary prepared by a contractor to the Panel.

Repeated Dose Toxicity

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >99.7%.
Method	
Method/guideline followed	Other
Test type	2-year carcinogenicity study.
GLP	Yes.
Year	1997.
Species	Rat.
Strain	Fisher 344.
Route of administration	Inhalation (vapor).
Duration of test	104 weeks.
Doses/concentration levels	0, 220, 700, or 7000 ppm.
Sex	50 male, 50 female per group.
Exposure period	6 hours/day.
Frequency of treatment	5 days/week for 104 weeks.
Control group and treatment	50 male, 50 female, exposed to air only.
Post exposure observation period	None.
Statistical methods	Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. Urine data was analyzed by nonparametric methods.
Test Conditions	Groups of 50 rats/sex /group (approx. 6 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for 104 weeks. Individual clinical observations were recorded initially, monthly through week 89, and then every 2 weeks until the end of the study. Individual body weights were recorded initially, monthly through week 91, and then every 2 weeks until the end of the study. Urine samples were collected 3, 6, 12, and 18 months from 10 rats/sex/group and analyzed for urine weight, creatinine, and vinyl lactic acid (a metabolite of isoprene). After 104 weeks of exposure, necropsies were performed on all rats and all major tissues preserved. Histopathologic examinations were performed on all tissues from all study animals. No blood analyses or organ weights were performed.
Results	
NOAEL (NOEL)	Not determined
LOAEL (LOEL)	Not determined
	Survival of all exposed groups was similar to the chamber controls. There were no exposure-related changes in clinical observations or body weights. The incidences of mammary gland fibroadenoma in 7,000 ppm males and in all groups of exposed females were significantly greater than those in the chamber control groups. The incidences of renal tubule adenoma in 700 and 7,000 ppm males and of renal tubule hyperplasia in 7,000 ppm males were significantly greater than those in the chamber controls. The severity of kidney nephropathy was slightly increased in 7,000 ppm males when compared to chamber controls. An exposure-related increase in the incidences of interstitial cell adenoma of the testis was observed in male rats. The incidences of bilateral interstitial cell adenoma and of unilateral and bilateral interstitial cell adenoma (combined) of the testis in 700 and 7,000 ppm males were significantly greater than those in the chamber controls. Single incidences of several rare neoplasms including benign astrocytoma, malignant glioma, malignant medulloblastoma, benign meningeal granular cell tumor, and meningeal sarcoma were observed in the brains of female rats in all three exposure groups. The incidences of splenic fibrosis in the 700 and 7,000 ppm males were significantly greater than that in the chamber control group.
Conclusions (contractor)	Isoprene exposures were associated with increases in rates of benign tumors in the testes and kidney (male), and mammary gland (male and female). No significant increases were seen for malignant tumors in this study. For this reason, and the fact that brain tumors in females were of several distinct cell types, the overall level of evidence presented for the

	carcinogenicity of isoprene in rats is, at most, limited.
Data Quality	
Reliabilities	1 - Reliable without restrictions,
References	National Toxicology Program (1997). Toxicology and Carcinogenesis Studies of Isoprene (CAS No. 78-79-5) in F344/N Rats (Inhalation Studies). Report No. TR-486.
Other	
Last changed	2 l-Aug-00 Robust summary prepared by a contractor to the Panel.

Repeated Dose Toxicity

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >99.0%.
Method	
Method/guideline followed	Other
Test type	2-year carcinogenicity study.
GLP	Yes.
Year	1996.
Species	Mouse.
Strain	$B6C3F_1$.
Route of administration	Inhalation (vapor).
Duration of test	105 weeks.
Doses/concentration levels	0, 10, 70, 140, 280, 700, 2200 ppm.
Sex	50 male, 50 female per group.
Exposure period	4 or 8 hours/day.
Frequency of treatment	Variable - 5 days/week for 20, 40, or 80 weeks.
Control group and treatment	50 male, 50 female, exposed to air only.
Post exposure observation period	Variable - animals held following exposures until week 96 or 105.
Statistical methods	Body weights, organ weights and hematology data were evaluated by analysis of variance (ANOVA) followed by Duncan's new multiple range test. Incidences of tumor types were analyzed using Fischer's exact test applied to each combination of exposure group and tumor type.
Test Conditions	Twelve groups of 50 male mice were exposed to 0, 10, 70, 140, 280, 700, or 2200 ppm for 4 or 8 hours/day, 5 days/week for 20, 40, or 80 weeks followed by a holding period until week 105. Three groups of 50 female mice were exposed to 0, 10, and 70 ppm for 8 hours/day for 80 weeks and also held for observation until week 105. Clinical observations and body weights were recorded weekly for 13 weeks and then monthly. Hematology and micronucleus evaluations were performed on 10 mice/group at 40 and 80 weeks. Complete histopathology evaluations were performed on organs and tissues from all mice.
Results	
NOAEL (NOEL)	10 ppm
LOAEL (LOEL)	70 ppm
	The carcinogenic potential of isoprene was evaluated as a function of concentration, length of daily exposure, and weeks of exposure as independent variables. Exposure of mice to the varied concentrations and schedules did not produce any significant signs of general toxicity. There was a concentration-related effect on survival due to increases in selected tumor development and associated mortality. Survival was near or below 50% after 95 weeks for mice exposed >280 ppm for 80 weeks; surviving mice in these groups were necropsied during week 96. Isoprene exposure caused an increase in neoplasms of the lung, liver, Harderian gland, forestomach, lymphoreticular system of male mice and in the Harderian gland and pituitary gland of female mice at concentrations of 70 ppm and higher. The product of concentration and length/duration of exposure was not a sufficient basis for prediction of tumor risk. In the micronucleus evaluation, the mean incidence of micronuclei in peripheral blood was significantly increased at 700 ppm and higher after 80 weeks, and at 2200 ppm after 40 weeks (the 280 and 700 ppm groups were not sampled by protocol design).
Conclusions (study authors)	The results of this study indicated that concentration, , length of daily exposure, and weeks of exposure did not affect tumor incidence equivalently and total cumulative exposure was not sufficient for predicting oncogenic risk from isoprene exposure in mice. There appeared to be threshold for oncogenic effects in mice, which varied by organ and tumor type. For male mice, the LOEL was 700ppm for lung tumor and hemangiosarcoma, 280

	ppm for malignant forestomach tumors and histiocytic sarcomas, 140 ppm for liver tuors, and 70 pmm for Harderian gland tumors. For female mice, the LOEL was 70 ppm for total non-liver, non-lung adenomas and possibly for hemagiosarcomas.
Data Quality	
Reliabilities	1 - Reliable without restrictions.
References	Placke ME, Griffis L, Bird M, Bus J, Persing RL, and Cox LA Jr (1996). Chronic inhalation oncogenicity study of isoprene in B6C3F1 mice. Toxicology 113:253-62.
Other	
Last changed	2 1 -Aug-00 Robust summary prepared by a contractor to the Panel.

Developmental Toxicity/Teratogenicity

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >99.7%.
Method	
Method/guideline followed	OECD 414
Test type	Developmental toxicity (teratogenicity) study.
GLP	Yes.
Year	1989.
Species	Rat and mouse.
Strain	Sprague-Dawley (rat) and CD-1/Swiss (mouse).
Route of administration	Inhalation (vapor).
Concentration levels	0,280, 1400, or 7000 ppm.
Sex	-30 pregnant females per group; plus 10 virgin females per group for comparison.
Exposure period	Gestation days 6-19 (rats) or 6-17 (mice).
Frequency of treatment	6 hours/day.
Control group and treatment	Air-exposed only.
Duration of test	Females sacrificed on gestation day 20 (rats) or 18 (mice).
Statistical methods	Not specified.
Test Conditions	Positively mated mice were exposed on days 6-17 of gestation and rats on days 6-19. The day of plug or sperm detection was designated as day 0. Body weights were recorded throughout the study period, and uterine and fetal body weights were obtained at sacrifice. Implants were enumerated and their status recorded. Live fetuses were sexed and examined for gross, visceral, skeletal, and soft-tissue craniofacial defects.
Results	
NOAEL maternal toxicity	7000 ppm (rats), 1400 ppm (mice).
NOAEL developmental toxicity	7000 ppm (rats), <280 ppm (mice).
Maternal effects	Exposure of pregnant rats to these concentrations of isoprene did not result in apparent maternal toxicity. The only effect observed in the rat dams was an increased kidney to body weight ratio at the highest level (7000 ppm). Exposure of Swiss (CD-1) mice to isoprene resulted in (from day 12 onward) significant reductions in maternal body weight, body weight gain during treatment, and uterine weight for the 7000 ppm group. Liver to body weight ratios for pregnant mouse dams were significantly increased in the 1400 and 7000 ppm groups compared to the control group, and kidney to body weight ratios were significantly increased the 7000 ppm group.
Embryo/fetal effects	In rats, there was no adverse effect on any reproductive index at any level and there was no increase in fetal malformations or variations. A slight, but not statistically significant, increase in the incidence of reduced vertebral ossifications (centra) was noted at 7000 ppm. In mice, there was an exposure-related and statistically significant reduction in fetal body weights at the 280 ppm level for female fetuses and at the 1400 ppm level for male fetuses. No embryotoxicity in the form of increased intrauterine death was present at any exposure level. There was no significant increase in the incidence of fetal malformations or variations, although two fetuses with cleft palate were found, one in each of the two highest exposure groups (1400 and 7000 ppm). Cleft palates were not detected in the control group. An increased incidence of supernumerary ribs was observed at 7000 ppm, although this skeletal variation is generally considered a secondary effect of maternal toxicity or stress and it's significance is unclear.
Conclusions (study authors)	Pregnant Sprague-Dawley rats and their offspring exhibited no significant toxic effects of isoprene at any exposure level in this study. Swiss (CD-1) mouse dams exhibited significant toxic effects only at the 7000 ppm level; however the offspring exhibited significant signs of toxicity, including reductions in fetal body weight at all exposure concentrations.

Data Quality	
Reliabilities	1 - Reliable without restrictions. NTP-sponsored study.
References	National Toxicology Program (1989). Inhalation Developmental Toxicology Studies: Teratology Study of Isoprene in Mice and Rats. TER88045; NTIS#DE89008095.
Other	
Last *changed*	2 1 -Aug-00 Robust summary prepared by a contractor to the Panel.

Toxicity to Reproduction

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >99%.
Method	
Method/guideline followed	Other.
Test type	13-week inhalation study.
GLP	Yes.
Year	1994.
Species	Rat and mouse.
Strain	F344 rats and B6C3F 1 mice.
Route of administration	Inhalation (vapor).
Duration of test	13 weeks.
Concentration levels	0, 70,700, or 7000 ppm.
Sex	10 male, 10 female per group.
Exposure period	6 hours/day.
Frequency of treatment	5 days/week.
Control group and treatment	10 male, 10 female, air-only exposed.
Statistical methods	Analysis of incidence of neoplastic and nonneoplastic lesions was performed.
Test Conditions	Groups of 10 animals /sex /group/species (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for thirteen weeks. Sperm motility, vaginal cytology, and histopathologic evaluations of the reproductive organs were performed on all rats and mice as part of the terminal sacrifice for the core 13-week subchronic inhalation study.
Results	
NOAEL	2200 ppm (rats). 220 ppm (mice).
	There were no exposure -related effects in rats except a slight increase in the incidence and relative severity of interstitial cell hyperplasia of the testis in the 7000 ppm group. In mice, testicular weight was reduced 35% in the 7000 ppm group, and morphological changes (seminiferous tubular atrophy) were detected in 2110 mice. Males in the 700 and 7000 ppm groups had 12% and 30% lower epididymal weights, 12% and 46% lower spermatid head counts, 12% and 46% lower sperm concentrations, and 6% and 23% reductions in sperm motility, respectively. The female mice exposed to 7000 ppm exhibited estrous cycle lengths significantly longer than the control group (4.8 vs. 4.2 days).
Conclusions	
(contractor)	No significant effects on reproductive endpoints were observed in rats except slight changes in the testis at the highest exposure level (7000 ppm). Mice exhibited significant effects at 700 ppm or higher, including increased estrous cycle length and testicular atrophy, and decreased epididymal weight, sperm head count, sperm concentration, and sperm motility.
Data Quality	
Reliabilities	2 - Reliable with restrictions. Limited reproductive toxicity data obtained as part of a NTP-sponsored subchronic inhalation toxicity study.
References	Melnick RL; Sills RC; Roycroft JH; Chou BJ; Ragan HA; Miller RA (1994). Isoprene, an endogenous hydrocarbon and industrial chemical, induces multiple organ neoplasia in rodents after 26 weeks of inhalation exposure. Cancer Res. 54:5333-5339.
Other	
Last changed	*2* 1 -Aug-00 Robust summary prepared by a contractor to the Panel.

Genetic Toxicity - in Vitro

Test Substance	2-Butene, 2-methyl. CAS# 5 13-35-9
Remarks	(2-methyl-2-butene, 85% purity)
Method	
Method/guideline followed	OECD 47 1
Type	Ames Salmonella/bacterial reverse mutation test (pre-incubation assay).
System of testing	Bacterial.
GLP	Yes.
Year	1980.
Species/Strain	*Salmonella typhimuriuml* TA98, TA100, TA1535, TA1537, TA1538
Metabolic activation	With and without.
Species and cell type	Rat liver S9 fraction.
Quantity	0.5 ml/plate.
Induced or not induced	Arochlor 1254-induced.
Concentrations tested	0, 0.2, 2, 20, 500, and 2000 ug/plate.
Statistical Methods	A positive response was defined as a minimum consistent doubling of the spontaneous reversion frequency, or if the number of induced revertants is less than twice the spontaneous rate then a reproducible, dose-related increase in any one strain/activation combination was interpreted as positive.
Test Conditions	The preincubation modification of the *Salmonella*/mammalian microsome assay was tested in five different *Salmonella* strains in the presence and absence of rat liver S-9. Five dose levels were tested, with three plates per dose level. Bacteria (0.5 ml) and S9 mix or pH 7.4 phosphate buffer (2.5 ml) were incubated at 37°C with the test substance in ethanol (0.1 ml) 30 minutes before incorporation of 0.5 ml of this mixture into 2 ml of top agar. Concurrent positive and solvent controls were also tested with and without metabolic activation. Two replicate assays were performed on different days to confirm the reproducibility of the results.
Results	
Genotoxic effects	Negative. The test substance was not mutagenic in any of the five strains of *Salmonella* tested in the presence or absence of metabolic activation (rat liver S9).
Conclusions (contractor)	The test substance was not mutagenic in the Ames Salmonella mutagenicity test.
Data Quality *Reliabilities*	1 - Reliable without restrictions.
Reference	Dean, B.J., Brooks, T.M., Hodson-Walker, G., and Hutson, D.H. (1985). Genetic toxicology testing of 41 industrial chemicals. Mutation Research 153:57-77.
Other	
Last changed	16-Oct-00 Robust summary prepared by a contractor to the Panel.

Genetic Toxicity - in Vivo

Test Substance	Isoamylene, CAS# 26760-64-5
Remarks	(90% 2-Butene, 2-methyl; 10% 1-Butene, 2-methyl).
Method	
Method/guideline followed	OECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes.
Year	1990.
Species	Mouse.
Strain	$B_6C_3F_1$
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0, 1034, 3258 or 10,350 ppm (analytical mean concentrations).
Exposure period	6 hours/day for 2 consecutive days.
No. of animals per dose	10 males/exposure level.
Control groups and treatment	10 males exposed to air (negative control), 10 males exposed to 1000 ppm 1,3 butadiene (positive control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Test Conditions	Ten male $B_6C_3F_1$ mice (weighing 22-26 g, approximately 8-9 weeks old) per group were exposed for 2 consecutive days, 6 h/day to 0, 1034, 3258, or 10,350 ppm (analytical means) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to normochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	The test substance induced a statistically significant ($p<0.01$) and dose-related increase in micronucleated PCEs at 3258 and 10,350 ppm. The mean micronucleated PCE values were 15.7 and 31.5 at 3258 and 10,350 ppm, respectively, compared to 2.6 micronucleated PCEs for the negative control and 4.6 at 1034 ppm. Statistically significant ($p<0.01$) and dose-related decreases in the mean percent PCEs, which is a measure of hematotoxicity, were also observed at 3258 and 10,350 ppm. The positive control produced a statistically significant increase in micronucleated PCEs (29.1).
Conclusions	
(study author)	Under the conditions of this study, inhalation exposure to 3258 and 10,350 ppm of the test substance induced statistically significant increases in micronucleated polychromatic erythrocytes in male $B_6C_3F_1$ mice.
Data Quality	
Reliability	1 - Reliable without restrictions.
References	ExxonMobil Biomedical Sciences, Inc. (1990). In Vivo Mammalian Bone Marrow Micronucleus Assay. Unpublished study.
Other	
Last changed	30-Aug-00 Robust summary prepared by a contractor to the Panel.

Genetic Toxicity - in Vivo

Test Substance	2-Butene, 2-methyl. CAS# 5 13-35-9
Remarks	(2-methyl-2-butene, >99.2% purity)
Method	
Method/guideline followed	OECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes
Year	1991.
Species	Mouse.
Strain	$B_6C_3F_1$
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0, 1005, 3207, or 9956 ppm (analytical mean concentrations).
Exposure period	6 hourslday for 2 consecutive days.
No. of animals per dose	10 males/exposure level.
Control groups and treatment	10 males exposed to air (negative control), 10 males exposed to 1000 ppm 1,3 butadiene (positive control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Test Conditions	Ten male $B_6C_3F_1$ mice (weighing 24-28 g, approximately 6-7 weeks old) per group were exposed for 2 consecutive days, 6 h/day to 0, 1005, 3207 or 9956 ppm (analytical mean) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to normochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	The test substance induced statistically significant ($p<0.01$) and dose-related increases in micronucleated PCEs at 3207 and 9956 ppm. The mean micronucleated PCE values were 4.2, 16.6 and 36.1 at 1005, 3207 and 9956 ppm, compared to 3.4 micronucleated PCEs for the negative control. A statistically significant ($p<0.01$) decrease in the mean percent PCEs, which is a measure of hematotoxicity, was also observed at 9956 ppm. The positive control produced a statistically significant increase in micronucleated PCEs (29.7).
Conclusions (study author)	Under the conditions of this study, inhalation exposure to 3207 and 9956 ppm of the test substance induced statistically significant increases in micronucleated polychromatic erythrocytes in male $B_6C_3F_1$ mice.
Data Quality *Reliabilities*	1 - Reliable without restrictions
References	ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay - Inhalation Dosing Method. Unpublished study.
Other	
Last changed	30-Aug-00
	Robust summary prepared by a contractor to the Panel.

Genetic Toxicity - in Vivo

Test Substance	2-Butene, 2-methyl. CAS# 5 13-35-9
Remarks	(2-methyl-2-butene, >99.2% purity)
Method	
Method/guideline followed	OECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes.
Year	1991.
Species	Rat.
Strain	CrlCDBR
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0, 1005, 3207, or 9956 ppm (analytical mean concentrations).
Exposure period	6 hours/day for 2 consecutive days.
No. of animals per dose	10 males/exposure level.
Control groups and treatment	10 males exposed to air (negative control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Test Conditions	Ten male CrlCDBR rats (weighing 295-345 g, approximately 9 weeks old) per group were exposed for 2 consecutive days, 6 h/day to 0, 1005, 3207 or 9956 ppm (analytical means) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to normochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	The test substance induced statistically significant ($p<0.01$) and dose-related increases in micronucleated PCEs at 3207 and 9956 ppm. The mean micronucleated PCE values were 4.2 and 4.9 at 3207 and 9956 ppm, respectively, compared to 2.7 for the negative control (air) and 2.2 at 1005 ppm. Statistically significant decreases in the mean percent PCEs, which is indicative of hematotoxicity, were also observed at all three exposure levels. Although the mean PCE frequencies at 1005, 3207 and 9956 ppm (48.62, 50.96, 49.76%, respectively) were slightly decreased form the negative control (54.86%), they were not different from each other and did not show evidence of a dose-response. Therefore, the biological significance of this observation is unclear.
Conclusions (study author)	Under the conditions of this study, inhalation exposure to 3207 and 9956 ppm of the test substance induced small but statistically significant increases in micronucleated polychromatic erythrocytes in male rats.
Data Quality Reliability	2 - Reliable with restrictions. No concurrent positive control was used.
References	ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay - Inhalation Dosing Method. Unpublished study.
Other	
Last changed	30-Aug-00 Robust summary prepared by a contractor to the Panel.

Genetic Toxicity - in Vivo

Test Substance	1 -Butene, 2-methyl CAS# 26760-64-5
Remarks	(2-methyl- 1 -butene, >99.2% purity)
Method	
Method/guideline followed	OECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes.
Year	1991.
Species	Mouse.
Strain	$B_6C_3F_1$.
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0, 1038, 3312, or 10,116 ppm (analytical mean concentrations).
Exposure period	6 hours/day for 2 consecutive days.
No. of animals per dose	10 males/ exposure level.
Control groups and treatment	10 males exposed to air (negative control), 10 males exposed to 1000 ppm 1,3 butadiene (positive control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Test Conditions	Ten male $B_6C_3F_1$ mice (weighing 24-30 g, approximately 7-8 weeks old) per group were exposed for 2 consecutive days, 6 h/day to 0, 1038, 33 12 or 10,116 ppm (analytical means) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to normochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	A dose-related increase in mean micronucleated PCEs was observed (2.4, 3.7, 3.6, and 4.6 at 0, 1038, 3312 and 10,116 ppm). However, since none of the exposed groups were statistically different from the negative control this finding was not considered to be biologically significant. The mean micronucleated PCE value of 4.6 at 10,116 ppm was slightly outside the normal range of the negative control (0-4), although it was not statistically significant ($p<0.09$). The mean percent of PCEs were within the normal range for all exposure groups. The positive control produced a statistically significant increase in micronucleated PCEs (43.1).
Conclusions (study author)	Under the conditions of this study, inhalation exposure to the test substance did not induce a statistically significant increase in micronucleated polychromatic erythrocytes in male $B_6C_3F_1$ mice.
Data Quality	
Reliability	1 - Reliable without restrictions.
References	ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay - Inhalation Dosing Method. Unpublished study.
Other	
Last changed	30-Aug-00 Robust summary prepared by a contractor to the Panel.

Genetic Toxicity - in Vivo

Test Substance	1-Butene, 2-methyl CAS# 26760-64-5
Remarks	(2-methyl-1-butene, >99.2% purity)
Method	
Method/guideline followed	OECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes.
Year	1991.
Species	Rat.
Strain	CrlCDBR
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0, 1038, 3312, or 10,116 ppm (analytical mean concentrations).
Exposure period	6 hours/day for 2 consecutive days.
No. of animals per dose	10 males/exposure level.
Control groups and treatment	10 males exposed to air (negative control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Test Conditions	Ten male CrlCDBR rats (weighing 337-4 14 g, approximately 10-l 1 weeks old) per **group were** exposed for 2 consecutive days, 6 h/day to 0, 1038, 33 12 or 10,116 ppm (analytical means) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to norrnochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	The test substance did not induce a statistically significant increase in micronucleated PCEs at 24 hours in any of the exposure groups. The mean percent PCEs were within the normal range of the negative controls.
Conclusions (study author)	Under the conditions of this study, inhalation exposure to the test substance did not induce a statistically significant increase in micronucleated polychromatic erythrocytes in male CrlCDBR rats.
Data Quality	
Reliability	2 ▪ Reliable with restrictions. No concurrent positive control was used.
References	ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay. Unpublished study.
Other	
Last changed	3 0-Aug-00 Robust summary prepared by a contractor to the Panel.

Genetic Toxicity ▪ in Vivo

Test Substance	Isoamylene, CAS# 26760-64-5
Remarks	(-92% 2-Butene, 2-methyl; -7% 1 -Butene, 2-methyl).
Method	
Method/guideline followed	OECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes.
Year	1991.
Species	Mouse.
Strain	$B_6C_3F_1$
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0, 1034, 3266 or 10,097 ppm (analytical mean concentrations).
Exposure period	6 hours/day for 2 consecutive days.
No. of animals per dose	10 males/exposure level.
Control groups and treatment	10 males exposed to air (negative control), 10 males exposed to 1000 ppm 1,3 butadiene (positive control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Test Conditions	Ten male $B_6C_3F_1$ mice (weighing 24-30 g, approximately 8-9 weeks old) per group were exposed for 2 consecutive days, 6 h/day to 0, 1034, 3266, or 10,097 ppm (analytical means) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to normochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	The test substance induced statistically significant ($p<0.01$) and dose-related increases in micronucleated PCEs at 3266 and 10,097 ppm. The mean micronucleated PCE values were 3.7, 22.6 and 42.1 at 1034, 3266 and 10,097 ppm, compared to 2.5 micronucleated PCEs for the negative control. Statistically significant ($p<0.01$) decreases in the mean percent PCEs, which is a measure of hematotoxicity, were also observed at 3266 and 10,097 ppm. The positive control produced a statistically significant increase in micronucleated PCEs (39.5).
Conclusions (study author)	Under the conditions of this study, inhalation exposure to 3266 and 10,097 ppm of the test substance induced statistically significant increases in micronucleated polychromatic erythrocytes in male $B_6C_3F_1$ mice.
Data Quality	
Reliability	1 - Reliable without restrictions
References	ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay. Unpublished study.
Other	
Last changed	30-Aug-00 Robust summary prepared by a contractor to the Panel.

Genetic Toxicity - in Vivo

Test Substance	Isoamylene, CAS# 26760-64-5
Remarks	(-92% 2-Butene, a-methyl; -7% 1 -Butene, 2-methyl).
Method	
Method/guideline followed	OECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes.
Year	1991.
Species	Rat.
Strain	CrlCDBR
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0, 1034, 3266 or 10,097 ppm (analytical mean concentrations).
Exposure period	6 hours/day for 2 consecutive days.
No. of animals per dose	10 males/exposure level.
Control groups and treatment	10 males exposed to air (negative control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Test Conditions	Ten male CrlCDBR rats (weighing 348-447 g, approximately 1 1- 12 weeks old) per group were exposed for 2 consecutive days, 6 h/day to 0, 1034, 3266, or 10,097 ppm (actual mean exposures) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to normochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	The test substance induced a statistically significant ($p<0.01$) increase in micronucleated PCEs at 10,097 ppm. The mean micronucleated PCE values were 3.4, 4.2, and 7.0 at 1034, 3266 and 10,097 ppm, compared to 3.3 micronucleated PCEs for the negative control. The slight increase in mean micronucleated PCEs (4.2) noted at 3266 ppm was slightly above the normal range for the negative control (O-4) although it was not statistically significant. The mean percent PCEs were within the normal range of the negative control for all exposed groups.
Conclusions (study author)	Under the conditions of this study, inhalation exposure to 10,097 ppm of the test substance induced a statistically significant increase in micronucleated polychromatic erythrocytes in male rats.
Data Quality	
Reliability	2 - Reliable with restrictions. No concurrent positive control was used.
References	ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay - Inhalation Dosing Method. Unpublished study.
Other	
Last changed	30-Aug-00 Robust summary prepared by a contractor to the Panel.

Acute Toxicity

Test Substance	Neohexene (3,3-dimethylbutene-1) CAS# 558-37-2 98.5% purity.
Method	
Method/guideline followed	OECD 40 1.
Type (test type)	Acute oral toxicity study.
GLP	Not specified.
Year	1982.
Species/Strain	Rat/Sprague-Dawley.
Sex	Male and female.
No. of animals per sex per dose	5/sex/group.
Vehicle	None.
Route of administration	Oral gavage.
Test Conditions	One group of five rats/sex was dosed orally at a level of 5000 mg/kg of body weight. The animals were observed at 1, 2, and 4 hours after dosing, and daily for a period of 14 days for mortality and signs of systemic toxicity. Body weights were recorded prior to treatment and at 7 and 14 days. The animals were necropsied at the end of the 14-day period and observed for gross abnormalities.
Results	
LD_{50}.	LD_{50} = >5 g/kg No animals died after dosing at 5000 mg/kg. Clinical signs of toxicity noted 1 hour after dosing included depression, soft feces, a hunched appearance, and rough fur coat. All animals appeared normal from Day 2 through termination of the study. All animals gained weight during the study. There were no significant findings at necropsy.
Conclusions	
(contractor)	The acute oral LD_{50} for the test substance was >5 g/kg.
Data Quality	
Reliability	1 - Reliable without restrictions.
References	Hazleton Laboratories America, Inc. (1982). Acute Oral Toxicity Study in Rats. Conducted for Phillips Petroleum Company, unpublished report.
Other	
Last changed	22-Aug-00 Robust summary prepared by a contractor to the Panel.

Acute Toxicity

Test Substance	Neohexene (3,3-dimethylbutene-1) CAS# 558-37-2 *98.5%* purity.
Method	
Method/guideline followed	OECD 403.
Type (test type)	Acute inhalation toxicity study.
GLP	Not specified.
Year	1982.
Species/Strain	Rat/Sprague-Dawley.
Sex	Male and female.
No. of animals per sex per dose	5/sex/group.
Vehicle	None.
Route of administration	Inhalation.
Test Conditions	One group of five rats/sex was placed in a 38 liter exposure chamber and exposed for four hours to the maximum practical vapor concentration. Analytical chamber concentrations were measured using a total hydrocarbon monitor (method or frequency not specified). The animals were observed hourly during the exposure and twice daily for a period of 14 days for mortality and signs of systemic toxicity. Body weights were recorded prior to treatment and at 2, 3, 4, 7, and 14 days. The animals were necropsied at the end of the 14-day period and observed for gross abnormalities.
Results	
LC_{50}	LC_{50} = >5 1,000 ppm. The mean analytical exposure concentration was 5 1,000 ppm. No animals died during the study. All the rats were observed prostrate in their cages during the exposure. All animals appeared normal throughout the post-exposure observation period. All animals gained weight during the study except the females at the Day 3 interval (slight group mean weight loss). There were no significant findings at necropsy.
Conclusions (contractor)	The acute inhalation LC_{50} for vapors of the test substance was >5 1,000 ppm.
Data Quality	
Reliability	1 - Reliable without restrictions.
References	Hazleton Laboratories America, Inc. (1982). Acute Inhalation Toxicity Test in Rats. Conducted for Phillips Petroleum Company, unpublished report.
Other	
Last changed	22-Aug-00 Robust summary prepared by a contractor to the Panel.

Acute Toxicity

Test Substance	Neohexene (3,3-dimethylbutene-1) CAS# 558-37-2 98.5% purity.
Method	
Method/guideline followed	Other.
Type (test type)	Respiratory tract irritancy study in mice.
GLP	Not specified.
Year	1982.
Species/Strain	Mouse/CD- 1.
Sex	Male
No. of animals per sex per dose	4/group.
Vehicle	None.
Route of administration	Inhalation.
Test Conditions	One group of four mice was placed in individual head-only plethysmographs attached to an exposure chamber. Baseline respiratory rates were first established with exposure to room air, then the mice were sequentially exposed to vapors of the test substance for 1 minute, next to room air for 10 minutes, then a second 1 minute vapor exposure, and finally a 5 minute room air recovery period. Individual respiratory rates and breathing patterns were recorded (methods not described). Group mean respiratory rates changes (% decrease) during exposure were presented; individual rates were categorized (0-25%, 25-50%, etc.). Analytical chamber concentrations were measured by a total hydrocarbon monitor.
Results	
RD_{50}	The RD_{50} (50% respiratory rate decrease) was greater than 55,000 ppm. The mean analytical exposure concentration was 55,000 ppm. Extremely slight decreases in respiration rates were noted 1 of 4 mice during the first one minute exposure and in 3 of 4 mice during the second exposure. The breathing patterns indicated slight upper airway irritancy only in 2 of the 4 mice during the second exposure. One mouse could not be evaluated during the first exposure due to excessive movement within the plethysmograph.
Conclusions	
(study author)	Based on these results, exposure to the vapors of the test substance at an analytical concentration of 5 1,000 ppm produced very slight upper airway irritancy in mice.
Data Quality	
Reliability	1 - Reliable without restrictions.
References	Hazleton Laboratories America, Inc. (1982). Respiratory Tract Irritancy Study in Mice. Conducted for Phillips Petroleum Company, unpublished report.
Other	
Last changed	22-Aug-00 Robust summary prepared by a contractor to the Panel.

Genetic Toxicity - in Vitro

Test Substance	Neohexene (3,3-dimethylbutene-1) CAS# 558-37-2 98.5% purity.
Method	
Method/guideline followed	OECD 47 1
Type	*Salmonella typhimurium* mammalian microsome plate incorporation assay (Ames Assay).
System of testing	Bacterial.
GLP	Not specified.
Year	1982.
Species/Strain	*Salmonella* / TA98, TA100, TA1535, TA1537, and TA1538.
Metabolic activation	With and without.
Species and cell type	Rat liver S9 fraction.
Quantity	0.5 ml/plate.
Induced or not induced	Arochlor 1254-induced (500 mg/kg for 5 days).
Concentrations tested	0, 32.3, 96.5, 289.5, 868.4, and 2605 ug/plate.
Control groups and treatment	Solvent control: dimethylsulfoxide (DMSO). Positive controls: N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG), 9-aminoacridine (9-AA), 2-nitrofluorene (2-NF), 2-aminoanthracene (2-AA).
Statistical Methods	A positive response was defined as a reproducible, dose-related increase in revertant colonies over three concentrations with the baseline increase twice the solvent control level.
Test Conditions	Five different *Salmonella* strains were tested in the presence and absence of rat liver S-9. The test substance was soluble in the solvent (dimethylsulfoxide, DMSO) at 100 mg/ml. Five dose levels were tested , with three plates per dose level. The maximum dose selected was 2605 ug/plate based on observed growth inhibition during an initial toxicity test. Concurrent positive controls were also tested with and without metabolic activation.
Results	
Genotoxic effects	Negative. The test substance was not mutagenic in any of the five strains of *Salmonella* tested in the presence or absence of Aroclor-induced rat liver S9.
Conclusions	
(study author)	The test substance was not mutagenic in the Ames Salmonella mutagenicity test.
Data Quality	
Reliabilities	1 - Reliable without restrictions.
Reference	Hazleton Laboratories America, Inc. (1982). *Salmonella typhimurium* mammalian microsome plate incorporation assay. Conducted for Phillips Petroleum Company, unpublished report.
Other	
Last changed	22-Aug-00 Robust summary prepared by a contractor to the Panel.

Genetic Toxicity - in Vitro

Test Substance	Neohexene (3,3-dimethylbutene-1) CAS# 558-37-2 98.5% purity.
Method	
Method/guideline followed	OECD 479.
Type	*In* vitro sister chromatid exchange (SCE) assay in Chinese hamster ovary cells.
System of testing	Chinese hamster ovary (CHO) cells.
GLP	Not specified.
Year	1982.
Metabolic activation	Aroclor 1254-induced Sprague-Dawley rat liver S9.
Concentrations tested	0, 1.3, 4.4, 13.2, 44, and 132 ug/ml.
Control groups and treatment	Solvent controls: dimethylsulfoxide (DMSO). Positive controls: ethylmethanesulfonate (without S9), cylcophosphamide (with S9).
Statistical Methods	Not specified.
Test Conditions	The test substance was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCE) both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and five doses of the test substance. The test substance was soluble in the solvent (DMSO) at 100 mg/ml. The maximum dose selected was 132 ug/plate based on observed growth inhibition in an initial toxicity study. Duplicate cultures were prepared for all dose levels and controls. Cells were exposed to the test substance for 2 hours, washed twice, and BrdU added to each culture. Cells were sampled 24 hours after BrdU addition; colcemid was added 2 hours prior to fixation. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level.
Results	
Genotoxic effects	Negative. No increases in SCEs were noted in cultured CHO cells treated with the test substance, with or without S9.
Conclusions (study author)	Under the conditions of this study, the test substance did not exhibit a positive response and is therefore considered not to be mutagenic in this test system.
Data Quality *Reliabilities*	1 - Reliable without restrictions.
Reference	Hazleton Laboratories America, Inc. (1982). *In vitro* sister chromatid exchange assay in Chinese hamster ovary cells. Conducted for Phillips Petroleum Company, unpublished report.
Other	
Last changed	22-Aug-00 Robust summary prepared by a contractor to the Panel.

Genetic Toxicity - in Vitro

Test Substance	Neohexene (3,3-dimethylbutene-1) CAS# 558-37-2
Test substance	98.5% purity.
Method	
Method/guideline followed	OECD 476.
Type	Mouse lymphoma forward mutation assay.
System of testing	Mammalian cell (mouse lymphoma cells/L5 178Y).
GLP	Not specified.
Year	1982.
Metabolic activation	With and without Arochlor-induced rat liver S9 mixture.
Concentrations tested	0, 82, 117, 168, 240, 343, 490, 700, and 1000 µl/ml.
Control groups and treatment	Ethylmethanesulfonate (EMS) was used as a positive control in the assays without S9 activation. 3-methylcholanthrene (MCA), which requires metabolic activation, was used as a positive control for assays with S9. The concurrent negative control was the vehicle (dimethylsulfoxide, DMSO).
Statistical Methods	A mutagenic response was defined as a dose-related response in two or more dose levels (in the absence of severe toxicity) with a greater than two-fold increase in the number of revertant colonies over the concurrent vehicle control value.
Test Conditions	Suspension cultures of mouse lymphoma cells, heterozygous for thymidine kinase activity, were grown in Fisher medium supplemented with 0.1% pluronic and 10% heat-inactivated horse serum (F1 OP) and exposed to the test substance in the same medium. Treated cells were grown for 48 hours to allow mutation expression. Approximately 500,000 cells from each culture were then plated in three selective media plates containing 2 ug/ml triflourothymidine (TFT) to select mutant clones. 100 cells from each culture were also seeded in non-selective plates without TFT to assess viability. The plates were incubated for approximately 12 days. The mutant colonies were counted on the selective (TFT) plates and the survivors on the non-selective (no TFT) plates.
Results	
Genotoxic effects	Positive. There was a slight increase in the induction of mutations without metabolic activation (maximum 2.7-fold increase). There were no significant increases with metabolic activation. The positive and negative controls responded in an appropriate manner.
Conclusions	Under conditions of this study, the test substance was weakly mutagenic in the mouse lymphoma assay without metabolic activation.
(contractor)	
Data Quality	
Reliabilities	1 - Reliable without restrictions.
Reference	Hazleton Laboratories America, Inc. (1982). Mouse lymphoma forward mutation assay. Conducted for Phillips Petroleum Company, unpublished report.
Other	
Last changed	22-Aug-00 Robust summary prepared by a contractor to the Panel